

**VITAMIN D AND CARDIOMETABOLIC DISEASE
RISK: A RCT AND CROSS-SECTIONAL STUDY**

Thesis submitted in accordance with the requirement of the
University of Chester for the degree of Doctor of Philosophy

By

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Declaration

“The material being presented for examination is my own work and has not been submitted for an award of this or another HEI except in minor particulars which are explicitly noted in the body of the thesis. Where research pertaining to the thesis was undertaken collaboratively, the nature and extent of my individual contribution has been made explicit.”

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30/01/2017

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Signature

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Date

To Papa, Faith, Preye and Ellen

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ABSTRACT

Given the strong evidence for a beneficial role of vitamin D in diabetes and CVD pathogenesis, and the prevalence of vitamin D deficiency, vitamin D supplementation has been advocated for the prevention of cardiometabolic disease.

To provide information on the effects of 5,000IU (125µg) vitamin D₃ on cardiometabolic risk, a double blind, RCT in a cohort of overweight and obese UK adult males with plasma 25(OH)D concentration < 75nmol/L for a duration of 8 weeks was conducted. To the best of my knowledge, this is the first RCT to investigate the effect of 5,000IU (125µg) vitamin D₃ on cardiometabolic markers in vitamin D insufficient, non-hypertensive and non-diabetic overweight and obese adult males.

Participants in the RCT were randomised to supplement their diet with either, 5,000IU (125µg) vitamin D₃ or placebo daily for 8 weeks. Primary outcomes were changes in endothelial function determined by plasma sE-selectin concentrations and arterial stiffness determined by pulse wave velocity (PWV) and augmentation index (AIx). Fifty-five overweight and obese participants were recruited with mean (±SD) age 34.5 ± 12 years, and plasma 25(OH)D concentration of 41.2 ± 17.7nmol/L. Although all study participants had suboptimal plasma 25(OH)D concentrations at baseline, vitamin D deficiency defined as 25(OH)D < 25nmol/L and < 30nmol/L was present in 22.2 % (n = 12) and 35.2 % (n = 19) of the study cohort, independent of treatment group.

After 8 weeks of daily vitamin D₃ supplementation, mean (±SD) plasma 25(OH)D concentration increased from baseline 38.4 ± 15.9 to 64.2 ± 19.5nmol/L, (*P* < 0.001) at week 4 and 72.8 ± 16.1nmol/L, (*P* < 0.001) at week 8 in the vitamin D group. The change in plasma 25(OH)D concentration was inversely associated with change in plasma glucose concentrations (*r* = -0.408, *P* = 0.048, as well as change in plasma HDL-C concentration (*r* = -0.503, *P* = 0.012) and positively associated with change in aortic central pulse pressure (*r* = 0.437, *P* = 0.033) at 4 weeks. Change in plasma 25(OH)D concentration post intervention was inversely associated with change in brachial augmentation index (*r* = -0.446, *P* = 0.029). The association between week 4

change in plasma 25(OH)D concentration with corresponding changes in PPao and plasma HDL-C concentration were absent at week 8.

There was a significant difference between vitamin D and placebo groups, in change in mean (\pm SD) brachial pulse pressure: -2.9 ± 3.4 vs. 1.6 ± 0.5 mmHg, ($P = 0.027$), however, no significant difference was observed between the vitamin D and placebo group in changes in mean (\pm SD) sE-selectin -10.8 ± 1.4 vs. -17.1 ± 2.9 ng/mL, ($P = 0.733$), PWV -0.1 ± 0.2 vs. -0.2 ± 0.1 m/s, ($P = 0.423$) and both brachial and aortic AIx, 15.4 ± 13.3 vs. $6.1 \pm 8.9\%$, ($P = 0.940$) and 3.1 ± 0.7 vs. $-0.2 \pm 3.2\%$, ($P = 0.705$) respectively, as well as the other cardiometabolic markers measured. Overall, the findings from this RCT do not support the hypothesis that a high daily dose of vitamin D reduces cardiometabolic disease risk in a cohort of non-hypertensive and non-diabetic overweight and obese adult males.

A second study was carried out to determine the prevalence of vitamin D deficiency and CVD risk in kidney stone patients. Vitamin D status and its association with CVD risk markers was assessed in a cohort of calcium oxalate kidney stones patients in the UK. To the best of my knowledge, this is also the first cross-sectional study carried out in calcium oxalate kidney stone patients, investigating the association between vitamin D status, bone health and selected CVD risk markers. Of the 24 calcium oxalate kidney stone patients recruited for this study, 3 (12.5%), 7 (29.2%) and 18 (75%) had serum 25(OH)D concentrations ≤ 25 nmol/L, <30 nmol/L and <50 nmol/L respectively. No significant associations were observed between serum 25(OH)D concentrations and CVD biomarkers. Although high prevalence of low serum 25(OH)D concentrations (75%) was observed in this study, this was not associated with the CVD biomarkers.

Interestingly, significant positive associations were observed between serum sE-selectin and calcium concentrations ($r = 0.53$, $P = 0.007$), serum hs-CRP and calcium concentrations ($r = 0.58$, $P = 0.003$). Furthermore, modest but significant inverse associations were observed between serum sE-selectin and HDL-C concentrations ($r = -0.49$, $P = 0.013$). Overall, no clear evidence for an association between serum vitamin D levels and cardiovascular disease risk factors was found

ABBREVIATIONS

1,25(OH) ₂ D	Calcitriol.
25(OH)D	25-hydroxy vitamin D
7DHC	7 dehydrocholesterol
AP-1	Activator protein 1
ADMA	Asymmetric dimethylarginine
AGEs	Advanced glycation end products
AIx,	Augmentation index
ALP	Alkaline phosphatase
ALTM	All - Laboratory Trimmed Mean
ARV	Antiretroviral.
BHF	British heart foundation
BMI	Body mass index
CAC	Coronary artery calcification
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
CONSORT	Consolidated Standards of Reporting Trials
CHD	Coronary heart disease
CKD	Chronic kidney disease
CRP	C-reactive protein
CYP2RI	Cytochrome P450 2RI
CYP24A1	Cytochrome P450 24A1
CYP27B1	Cytochrome P450 27B1
CYP3A4	Cytochrome P450 3A4
CV	Coefficient of variation
CVD	Cardiovascular disease
C/EBP α	CCAAT/enhancer binding protein alpha
DBD	DNA binding domain
DBP	Diastolic blood pressure
DH	Department of health
DEQAS	Vitamin D External Quality Assessment Scheme

DNA	Deoxyribonucleic acid
DRV	Dietary reference values
EAR	Estimated average requirement
ECM	Extracellular matrix
ED	Ejection duration
eGFR	Estimated glomerular filtration
ELISA	Enzyme linked immunosorbent assay
EFSA	European Food Safety Authority
EPCs	Endothelial progenitor cells
EDV	Endothelial dependent vasodilation
FAS	Fatty acid synthase
FGF-23	Fibroblast growth factor 23
FMD	Flow mediated dilatation
FSM	Fat soluble micro constituents
FXR	Farnesoid X receptor
GDM	Gestational diabetes mellitus
GIT	Gastrointestinal tract
GLUT	Glucose transporter
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HD	Haemodialysis
HDL-C	High density lipoprotein cholesterol
HIV	Human immunodeficiency virus
HOMA-IR	Homeostatic model assessment- insulin resistance
HUVEC	Human umbilical vein endothelial cells
HR	Heart rate
hs-CRP	High sensitivity-C-reactive protein
IL	Interleukin
IOM	US Institute of Medicine
IFN- γ	Interferon gamma
IOM	Institute of Medicine
LBD	Ligand binding domain
LDL-C	Low density lipoprotein cholesterol
MDA	Malondialdehyde

MAP	Mean arterial pressure
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemo attractant protein-1
MI	Myocardial infarction
MIF	Macrophage migration inhibitory factor
NADPH	Nicotinamide adenine dinucleotide phosphate
NC	Nephrocalcin
NDNS	National Diet and Nutrition Survey
NFAT	Nuclear factor of activated T-cells
NFkB	Nuclear factor -kappaB
NGAL	Neutrophil gelatinase associated lipocalin
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
Nmol/l	Nanomoles per litre
NO	Nitric oxide
NOS	National Osteoporosis Society
OPN	Osteopontin
PAD	Peripheral artery disease
PHE	Public Health England
PIS	Participant information sheet
PP	Pulse pressure
PPao	Aortic pulse pressure
PPAR γ	Peroxisome proliferator- activated receptor gamma
PRA	Plasma renin activity
PTG	Parathyroid gland
PTH	Parathyroid hormone
PWV	Pulse wave velocity
RAS	Renin-Angiotensin System
RDA	Recommended daily allowance
ROS	Reactive oxygen species
RCT	Randomised controlled trial
RHI	Reactive hyperaemia index
RH-PAT	Reactive hyperaemia peripheral arterial tonometry.

RT	Return time
RUNX2	Runt-related transcription factor 2
RXR	Retinoid X receptor
SACN	Scientific Advisory Committee of Nutrition
SBP	Systolic blood pressure
SDMA	Symmetric-dimethylarginine
SLE	Systemic lupus erythematosus
SBPao	Aortic central systolic blood pressure
SWL	Shock wave lithotripsy
sICAM-1	Soluble intracellular molecule-1
sVCAM-1	Soluble vascular cell adhesion molecule-1
TAG	Triacylglycerol
THP	Tamm-Horsefall protein
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
Th,	T helper
THP	Tamm-Horsfall protein
TLR	Toll like receptors
TNF- α	Tumor necrosis factor alpha
T2DM	Type 2 diabetes mellitus
TRPV6	Transient Receptor Potential Cation Channel Subfamily V Member 6
UK	United Kingdom.
UTI	Urinary tract infections
UTPF-1	Urinary prothrombin fragment 1
UVB	Ultra violet B radiation
UTR	Untranslated
VDR	Vitamin D receptor
VDRE	Vitamin D response element
VDBP	Vitamin D binding protein
VEGF	Vascular endothelial growth factor
Vitamin D ₂	Ergocalciferol

Vitamin	Cholecalciferol
D ₃	
VLDL-C	Very-low density lipoproteins-C
VSMC	Vascular smooth muscle cell
vWF	Von willebrand factor
WC,	Waist circumference

1.0 GENERAL INTRODUCTION

1.0 GENERAL INTRODUCTION

1.1 VITAMIN D

Vitamin D is a fat soluble secosteroid hormone which imparts both endocrine and autocrine functions (Vanchinathan & Lim, 2012). The principal endocrine function of vitamin D is supporting calcium and phosphorus homeostasis, which is achieved through the modulation of intestinal/kidney calcium absorption (Lee, O'Keefe, Bell, Hensrud, & Holick, 2008), and resorption from the bones (Pfeifer, Begerow, & Minne, 2002). Vitamin D also performs non-skeletal functions due to the presence of vitamin D receptors (VDR) in the nucleus of many tissues and cells of the human body, including cells of the parathyroid gland, skin, immune system, colon, pituitary gland, ovary, breast, prostate, liver, pancreas, heart, vascular smooth muscle endothelial cells and skeletal muscle amongst others (Brewer, Michos, & Reis, 2011; Holick, 2007). Studies have shown that several hundred genes throughout the body are being regulated by the VDR directly and indirectly in order to modulate cellular differentiation, proliferation, angiogenesis and apoptosis (Nagpal, Na, & Rathnachalam, 2005). Changes in the nucleotide sequence of a gene that is distinct to a particular cell type expressing the VDR can be described as the autocrine function of vitamin D. Some of the autocrine effects of vitamin D include its role in cancer, immune system and cardiovascular disease (CVD) amongst others. Vitamin D exerts these beneficial effects by promoting and obstructing cell reproduction, programmed cell death, and the modulation of inflammatory pathways (Cohen-Lahav, Douvdevani, Chaimovitz, & Shany, 2007; Heaney, 2008).

1.2 FORMS OF VITAMIN D

There are two important natural forms of vitamin D, vitamin D₂ or ergocalciferol (C₂₈H₄₄O) with a molecular mass of 396.65g/mol (**Fig.1.1**) and vitamin D₃ or cholecalciferol (C₂₇H₄₄O) with a molecular mass of 384.64g/mol (**Fig.1.2**). Vitamin D₂ is a 28 carbon molecule formed in plants and fungi by the ultra violet (UV) irradiation of the plant sterol ergosterol whilst vitamin D₃ is a 27 carbon molecule produced in the human skin from exposure to the sun (Holick, 2006). The major difference between vitamin D₂ and D₃ is the structure of the side chain to the sterol skeleton, which consists of an additional methyl group on carbon 24 and a double bond between carbon atoms 22 and 23 (Houghton & Vieth, 2006; Norman, 2008).

Although both forms have been reported to have similar molecular actions, vitamin D₃ has been reported to be more potent compared to vitamin D₂ regarding its bioavailability and the maintenance of vitamin D status (Armas, Hollis, & Heaney, 2004; Heaney, Recker, Grote, Horst, & Armas, 2011; Lehmann et al., 2013). A systematic review and meta-analysis performed by (Tripkovic et al., 2012) showed that vitamin D₃ was significantly more effective at increasing serum 25(OH)D concentrations compared with vitamin D₂ ($P = 0.001$) in 8 out of 10 RCTs identified, thus vitamin D₃ maybe a better choice for supplementation. There is evidence however, that vitamin D₂ is as efficient as D₃ in maintaining serum 25(OH)D₃ concentrations (Biancuzzo et al., 2010; Holick et al., 2008).

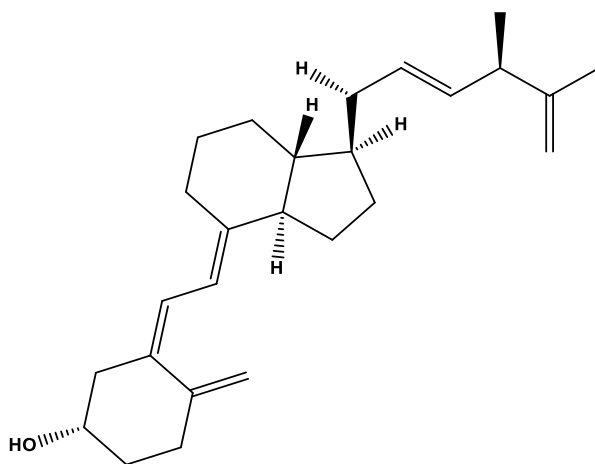


Fig.1.1 Molecular structure of ergocalciferol (D₂)

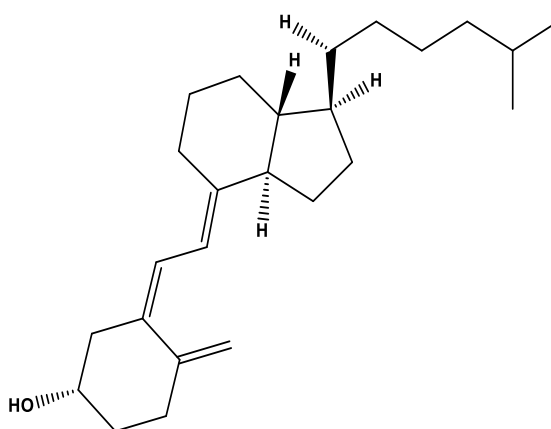


Fig.1.2. Molecular structure of cholecalciferol (D₃)

1.3 SOURCES OF VITAMIN D

Vitamin D is a fat-soluble micronutrient and the primary source of this vitamin is through sunlight synthesis, dietary intake, supplements and fortified foods are secondary sources of vitamin D.

1.3.1 Sunlight irradiation

Exposure of the skin to sunlight provides most of the vitamin D requirements for humans (Holick, 2004), thus, an individual increased sun exposure leads to improved vitamin D status. Vitamin D is synthesised by the skin following sunlight irradiation between 1000 h and 1500 h during summer and spring (Holick, 2007; Moan, Porojnicu, Dahlback, & Setlow, 2008). Vitamin D synthesised by the skin is more likely to last for an extended period in the blood compared with ingested vitamin D (Haddad, Matsuoka, Hollis, Hu, & Wortsman, 1993). The amount of vitamin D produced when an uncovered human adult skin is exposed to one minimal erythema dose of UV radiation (a slight pinkness to the skin 24 h after exposure), is equivalent to ingesting between 10,000 IU (250µg) and 25,000 IU (625µg) vitamin D (Holick & Chen, 2008). Vitamin D synthesis by UVB irradiation is discussed in detail in section 1.7.1.

1.3.2 Dietary sources

Diet is a secondary source of vitamin D and plays a limited role in vitamin D status, as few foods naturally contain vitamin D (Lu et al., 2007). Animal food products and fortified foods are the main dietary sources of vitamin D in the UK (Schmid & Walther, 2013). Vitamin D is naturally more abundant in animal and fish products compared to plant products. In a cross-sectional analysis of 2107 White men and women (1388 meat eaters, 210 fish eaters, 89 vegans and 420 vegetarians), Crowe et al., (2011), reported reduced plasma 25(OH)D concentrations in both vegetarians and vegans compared to meat and fish eaters in a UK study. Vitamin D₃ and its precursor, 7 dehydrocholesterol (7DHC), a derivative of cholesterol has been found to occur in microalgae and in the leaves of plants including vegetables such as potato, tomato and pepper, even though their vitamin D content differs and there is lack of evidence whether the edible portions also contain vitamin D₂ (Japelt & Jakobsen, 2013). It is important to note that the vitamin D content of oily fish varies

widely within species and farmed salmon have been found to contain about one quarter of the vitamin D found in wild salmon. Wild mushroom, a rich dietary source of vitamin D₂ (13-30µg/100g), also contains more vitamin D₂ than cultivated mushrooms (which are grown in the dark) (Teichmann, Dutta, Staffas, & Jägerstad, 2007). UK Dietary surveys in White non-breastfed infants and very young children, found formula milk to be the main contributor to dietary vitamin D status, as it provides an average of 85% vitamin D intake for children aged 4–6 months. In the UK, formula milk contributed 80% and 72% vitamin D for infants aged 7–9 months and 10–11 months respectively, and 29% for children aged 12–18 months (Lennox, Sommerville, Ong, Henderson, & Allen, 2013). For children aged 1.5–3 years and 4–10 years milk and milk products provided 24 and 13% respectively of vitamin D intake (Bates et al., 2014). The vitamin D content of the major dietary sources of vitamin D is shown in Table 1.1.

Table 1.1. Vitamin D content of foodstuffs (DH, 2013 and SACN, 2016)

Food source	Vitamin D (µg/100g)
Fish	
Mackerel raw	8.0
Salmon, raw	5.9
Sardines raw	4.0
Yellow fin tuna raw	3.2
Cod chilled/frozen, raw	Trace
Prawns, king, raw	Trace
Meat	
Pork leg joint, raw	0.9
Lamb chop, raw	0.8
Liver (lamb, fried)	0.9
Liver (calf, fried)	0.3
Beef (rump steak, fried)	0.7
Eggs	
Eggs (whole boiled)	3.2
Eggs (yolk, boiled)	12.6
Fortified foods	
Bran flakes	4.2
Corn flakes	4.2
Rice cereal	4.2
Fat spreads (reduced fat 62-75% polyunsaturated)	7.5

1.4 VITAMIN D FOOD FORTIFICATION

The National Diet and Nutrition Survey report shows that over one-fifth of the UK population had circulating serum 25(OH)D concentration < 25 nmol/L (Bates et al., 2014). This low concentration occurs mainly in people (particularly children) with reduced exposure to the sun or poor dietary intake of vitamin D. Fortification with vitamin D of some foodstuffs has been accepted as a strategy to improve serum 25(OH)D concentrations, however, mandatory and voluntary fortification differs

between countries. In the UK, it was mandatory to fortify margarine with 7.05 µg and 8.82 µg vitamin D from 1940, until this requirement was removed in 2013 as part of the Government's Red Tape Challenge, with the aim of reducing the overall burden of regulation (SACN, 2016). Nevertheless, infant formulas are still mandatorily fortified and most margarine, fat spreads, powdered or evaporated milk and some breakfast cereals are fortified with vitamin D on a voluntary basis (SACN, 2015). Findings from a systematic review and meta-analysis of 16 randomised controlled trials (RCTs) worldwide showed that vitamin-D fortified foods raised 25(OH)D concentrations in a dose-dependent manner. The average daily individual intake of approximately 11 µg vitamin D (3–25 µg/day) from fortified foods raised serum 25(OH)D by 19.4 nmol/L equivalent to a 1.2 nmol/L increase for each 1 µg ingested (Black, Seamans, Cashman, & Kiely, 2012).

In Canada, milk and margarine are fortified mandatorily with 1µg/100ml and 13.3µg/100g of vitamin D respectively (Health Canada, 2014). In the US, 75% of ready to eat breakfast cereals, almost all liquid milk, 8-14% of cheeses, juices, and spreads, approximately a quarter of yoghurts, and slightly more than half of all milk substitutes are fortified with vitamin D (Yetley, 2008). Since 2007, Sweden has adopted a policy of mandatory fortification of milk (in the range 3.8–5.0 µg/L) and margarines and fat spreads were fortified with vitamin D in the range of 7.5–10 µg/100 g (Helidan, Kosonen, & Tapanainen, 2013). Vitamin D fortification of liquid milk products (0.5µg/dL milk) and margarines (10µg/100g) were introduced in Finland in February 2003. A major drawback of vitamin D fortification of milk is that it would not be beneficial to individuals with lactose intolerance and non-consumers of fortified milk (Spiro & Buttriss, 2014).

1.5 VITAMIN D SUPPLEMENTATION

Vitamin D supplements contain either D₂ or D₃ and are present in a wide range of licensed medicines and food supplements including calcium supplements and fish oil products, which contain up to 500 IU (12.5µg). Multivitamins which contain 400 IU (10µg) are readily available in capsules and tablets and oil preparations that contain 100,000 IU (2,500µg) (Holick, 2005).

1.6 VITAMIN D INTAKE IN THE UK

The mean dietary vitamin D intake from all sources including dietary supplements in breast fed and non-breast fed infants and the general UK populations are shown in Table 1.2

Table 1.2. Mean dietary vitamin D intake for the UK population (SACN, 2016)

Age groups	Mean intake in breast fed infants (µg/day)	Mean intake in non-breast fed infants (µg/day)	Mean intake in young children/adults (µg/day)	RNI (µg/day)
4-11m	3.5-3.8	7.7- 10		8.5-10µg
12-18 m	2.6 -	3.9		10µg
1.5-18Y	-	-	2.3-2.7	10µg
19-65+	-	-	3.6-5.1	10µg

1.7 VITAMIN D METABOLISM

Vitamin D is synthesised endogenously (sunlight exposure) and exogenously (dietary intake).

1.7.1 Synthesis in the skin from UVB irradiation

Exposure of the basal and supra-basal layer of the epidermis of the skin to UVB light at a wavelength of 290-315nm, induces the photolytic conversion of 10-15% 7-DHC also referred to as provitamin D₃, present on the epidermis and dermis of the skin (Lehmann & Meurer, 2010). Photolysis causes isomerisation, which results in the activation of double bonds between C-9 and C-10, leading to the opening up of the B rings. The B rings rearrange to form 9, 10 secoesterol, a steroid with a broken ring also known as previtamin D₃, a thermodynamically unstable isomer. Previtamin D₃ undergoes non-enzymatic thermal isomerisation, which is a temperature-sensitive rearrangement of three double bonds to form the biologically inactive vitamin D₃ (cholecalciferol) (**Fig.1.3**). This process occurs in the plasma membrane of both the dermis and epidermis over the duration of three days (Holick, 2010). It has been reported that 50% of previtamin D₃ is capable of isomerising to vitamin D₃ within

2.5 hours in the skin, explaining the rapid increase in serum levels of vitamin D₃ on exposure to UVB (Holick, MacLaughlin, & Doppelt, 1981). It is important to note that, increased UVB exposure, does not lead to the production of more previtamin D₃, but instead, it is converted to biologically inactive metabolites such as lumisterol, pyrocalciferol, and tachysterol, to prevent the build-up of vitamin D₃ at toxic levels. Cutaneous vitamin D₃ is reversibly photo-converted and isomerised into suprasterol I and II and to 5, 6-trans-vitamin D₃ which are made available when previtamin D₃ concentrations fall (Hosseini-nezhad & Holick, 2013; Norval, Bjorn, & de Gruijl, 2010).

1.7.1.1 Hydroxylation to 25(OH)D₃

As fat soluble molecules with low aqueous solubility, vitamin D metabolites must be transported bound to plasma proteins such as the vitamin D binding protein (VDBP) (Dusso, Brown, & Slatopolsky, 2005). Before inactive vitamin D₃ undergoes hydroxylation, it must first bind to the VDBP, which transports inactive D₂ and D₃ through blood and lymph to the liver. In the liver, they are hydroxylated at position C-25 by an enzyme CYP2RI, also known as 25-hydroxylase to produce 25 hydroxy-vitamin D₃ (25(OH)D₃) also known as calcidiol (**Fig.1.3**). The 25 - hydroxylation has also been shown to occur in some extra- hepatic tissues such as, cardiomyocytes, endothelium, vascular smooth muscle and pancreatic β cells amongst others (DeLuca, 2008). The primary circulating form used in establishing an individual's serum/plasma vitamin D status is 25(OH)D, as it has a circulating half-life of 15 days (Byrdwell et al., 2008). The use of 25(OH)D as the ideal marker of vitamin D status was as a result of its association with the risk of hip fracture (Lips, 2007). The hydroxylation of vitamin D₃ to 25(OH)D₃ in the liver has little or no regulation.

1.7.1.2 VDBP

VDBP is an α -globulin, 58kDa glycoprotein belonging to the serum albumin superfamily, it is a binding protein for all vitamin D metabolites in the serum, although it has 20 times more binding affinity for 25(OH)D₃ compared to active vitamin D (Christakos, Dhawan, Verstuyf, Verlinden, & Carmeliet, 2016). This indicates that, it may significantly influence 25(OH)D-mediated intracrine responses (Chun et al., 2014). VDBP binds to fatty acids and acts as a chemical stimulus that attracts macrophages to site of inflammation, as it is capable of recruiting neutrophils

(Chun, 2012; Trujillo et al., 2013). This is supported by evidence that neutrophil recruitment is hindered in VDBP null mice. Additionally, there are noticeable reductions in serum $1,25(\text{OH})_2\text{D}_3$ and $25(\text{OH})\text{D}_3$ concentrations in VDBP null mice even though serum PTH, calcium and phosphorus concentrations remains normal (Safadi et al., 1999). Hence, maintenance of normal serum concentrations of calcium may be attributed to the ability of the VDR to concentrate vitamin D in tissues due to its high affinity for vitamin D resulting in the upregulation of calcium homeostasis maintenance genes.

VDBP bound vitamin D metabolites have a longer circulating half-life, as they are at minimal risk of hepatic metabolism and biliary excretion. Unbound metabolites are incapable of reaching target cells and are hence, unable to exert biological activity. VDBP has been reported to prevent toxicity by buffering the free concentrations of active form of vitamin D (Christakos et al., 2016). Active vitamin D concentration does not regulate VDBP, as its concentrations are reduced by malnutrition, renal and hepatic disease, and are increased during pregnancy and estrogen therapy (Dusso et al., 2005). VDBP is greatly polymorphic, and this affects its protein function as shown in 3 common variants: *GC1F*, *GC1S*, and *GC2*. The prevalence of these polymorphisms is influenced by race (Engelman et al., 2008). Blacks and Asians carry the polymorphisms in Gc1f, whereas whites carry the Gc1s form of DBP. The Gc2 form rarely occurs in blacks but is more prevalent in people of European and Asian ethnicity (Christakos et al., 2016). Polymorphism in the VDBP amino acid sequence alters the binding affinity of VDBP for vitamin D ligands, Gc1f and Gc2 have the highest and lowest affinity respectively (Bouillon, van Baelen, & de Moor, 1980). Circulating concentrations of $25(\text{OH})\text{D}$ are associated with polymorphisms of Gc1s and Gc2 as reported by genome-wide association studies (Ahn et al., 2010; Wang et al., 2010). Associations with reduced $25(\text{OH})\text{D}_3$ concentrations have been reported in TT carriers for Gc1s and AA carriers for Gc2. These polymorphisms may affect the bioavailability of vitamin D (Chun et al., 2014).

.7.1.3 Hydroxylation to $1, 25(\text{OH})_2 \text{D}_3$

VDBP binds to $25(\text{OH})\text{D}_3$ forming a complex that is transported to the kidneys with the aid of megalin for glomerular filtration. Megalin, a 600-kDa transmembrane protein and cell surface receptor for VDBP, stimulates $25(\text{OH})\text{D}_3$ absorption in the

tubular epithelial cells through endocytosis (Chun et al., 2014). The ability of megalin to effect renal absorption of $25(\text{OH})\text{D}_3$ is noteworthy, as mice deficient in megalin, have been shown to lose $25(\text{OH})\text{D}_3$ and VDBP complex in the urine and also have defects of bone metabolism (Nykjaer et al., 1999). A cytoplasmic adaptor protein disabled 2 (dab 2), works together with megalin to enable the cellular uptake of VDBP/ $25(\text{OH})\text{D}_3$ in the kidneys by triggering the exact routing of megalin by binding to the cytoplasmic tail of megalin (Morris, Tallquist, Rock, & Cooper, 2002; Willnow & Nykjaer, 2010). Cubulin, present in the proximal tubule, is another cell surface receptor for VDBP, it works together with megalin to internalise the $25(\text{OH})\text{D}_3$ and VDBP complex (Nykjaer et al., 2001). On reaching the proximal renal tubules of the kidneys, $25(\text{OH})\text{D}_3$ undergoes final hydroxylation at position C-1 of the A ring, resulting in the formation of the biological and functional active form called $1,25(\text{OH})_2\text{D}_3$ or calcitriol. This process is catalysed by 1α hydroxylase (mitochondrial CYP2B1) (**Fig.1.3**). It is important to note that the active form is not a good indicator of vitamin D status as a result of its presence in circulation only in picomolar quantities and it has a short circulating half-life of 15 hours (Deeb, Trump, & Johnson, 2007; Jones, 2008). The mitochondrial 1α hydroxylase enzyme CYP27B1 is significant, because despite regular vitamin D consumption, vitamin D dependency rickets type 1 (pseudovitamin D deficiency rickets) have been reported to occur following mutations which results in deleted or inactive CYP27B1 (Kitanaka et al., 1998). The metabolism of $25(\text{OH})\text{D}_3$ depends on the calcium needs of an individual. An increased need for calcium results in 1α hydroxylation, whilst excess calcium leads to 24-hydroxylation (DeLuca, 2008).

1.7.1.4 Inactivation of $25(\text{OH})\text{D}_3$

In addition to the conversion of $25(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ in the kidneys by CYP27B1, $25(\text{OH})\text{D}_3$ can also be inactivated by CYP24A1, a 24 hydroxylase, through its conversion to 24, $25(\text{OH})_2\text{D}_3$ by hydroxylation at position C-24 yielding calcitroic acid or $1,25(\text{OH})_2\text{D}_3$ -26,23S-lactone (**Fig.1.3.**) (Carlberg & Molnár, 2012). CYP24A1 is a mitochondrial inner membrane cytochrome P-450 enzyme present in all cells containing the VDR. CYP24A1 modulates the circulating levels of $1,25(\text{OH})_2\text{D}_3$, following increased serum concentration by catalysing the conversion of $1,25(\text{OH})_2\text{D}_3$ into 24-hydroxylated products targeted for excretion or by forming $24,25(\text{OH})_2\text{D}_3$ thereby reducing the concentration of $25(\text{OH})\text{D}_3$ available for 1α -

hydroxylation. In addition, it regulates $1,25(\text{OH})_2\text{D}_3$ concentration within cells, leading to correct cellular response (Jones, Prosser, & Kaufmann, 2012). It is important to note that $1,25(\text{OH})_2\text{D}_3$ analogues (synthetic analogues of $1,25(\text{OH})_2\text{D}_3$, most of which are modified in their aliphatic side chain, are used to improve the biological profile of $1,25(\text{OH})_2\text{D}_3$ for therapeutic purposes) are metabolised by same CYP enzymes, however, the efficiency with which this is done is varied resulting in different metabolic profiles (Carlberg & Molnar, 2012; Verstuyf, Carmeliet, Bouillon, & Mathieu, 2010).

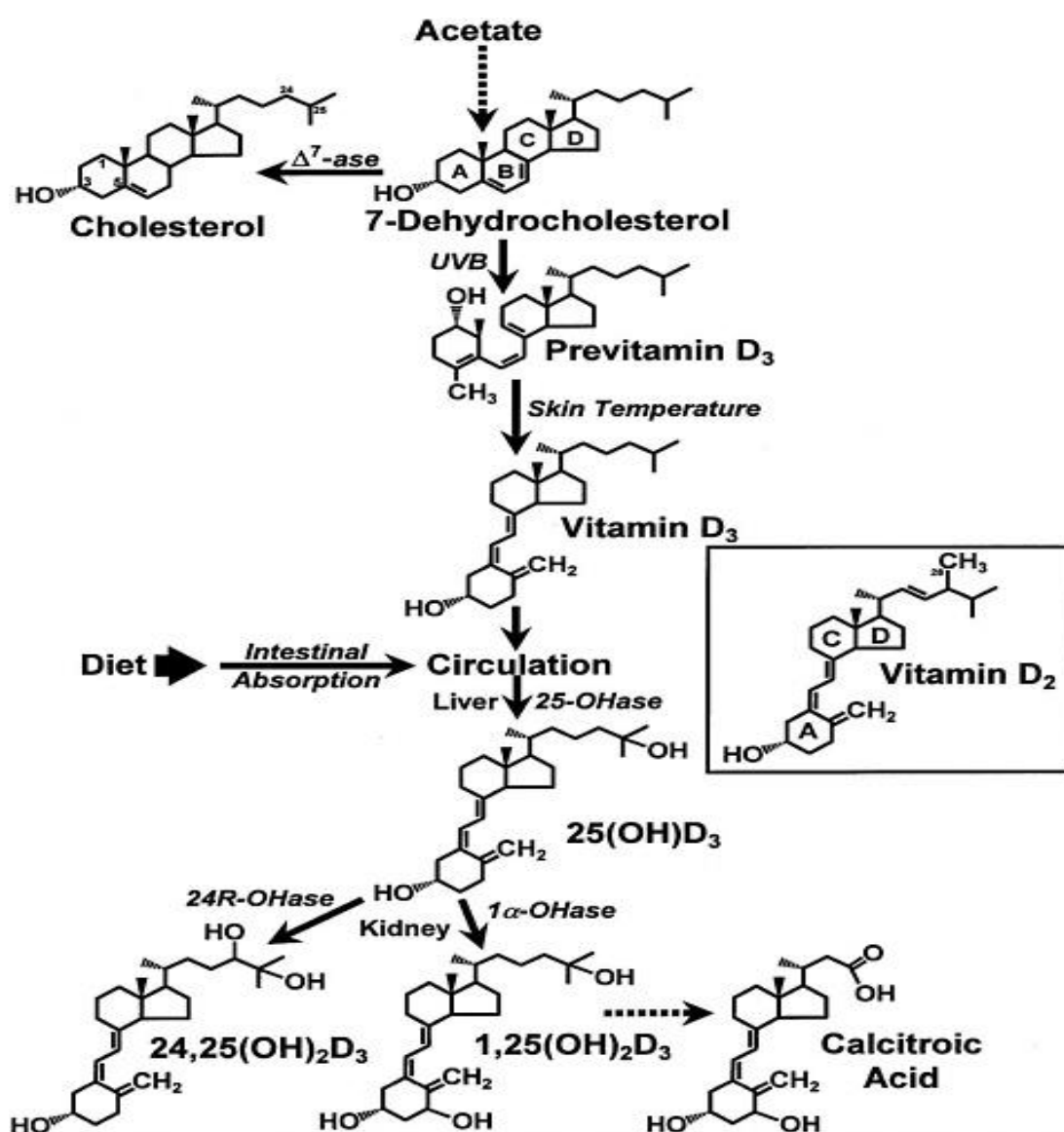


Fig.1.3. Metabolic pathway of vitamin D synthesis (Holick, 1996).

1.7.2 Factors affecting cutaneous vitamin D₃ synthesis

Prior to interacting with 7DHC, levels of UVB may be reduced by several factors which affect skin synthesis of vitamin D₃ and they include: zenith angle, latitude, season, time of the day, sunscreen use/protective clothing, temperature, exposure to radiation and the activity of 7DHC (Lehmann & Meurer, 2010).

1.7.2.1. Zenith angle, season and latitude

Approximately 1% of UVB radiation reaches the earth's surface even in summer as the stratospheric ozone layer absorbs approximately 99% of UVB radiation, hence living at a latitude above 33° during winter produces little or no vitamin D₃ from sun exposure from the months of November through to February (Caldwell & Flint, 1994; Ladizesky et al., 1995; Webb, Kline, & Holick, 1988). The zenith angle of the sun is more oblique and is comparable to winter sun in the early mornings and late afternoons and as a result the best time for the synthesis of vitamin D₃ is between 10 am and after 3pm (Holick, 2010). Seasons of the year also affect vitamin D₃ synthesis. A study aimed at determining the prevalence of hypovitaminosis D in 7437 white British people found it to be highest during winter and spring. However, circulating 25(OH)D peaked in September (Hypponen & Power, 2007). The higher the altitude, the shorter the path length travelled by UVB radiation in the atmosphere leading to increased previtamin D₃ synthesis (Holick, Chen, Lu, & Sauter, 2007).

1.7.2.2 Air pollutants, glass windows, sunscreen use and skin thickness

Air pollutants including nitrous oxide and ozone which are common in large cities absorb solar UVB radiation thus, reducing the normal synthesis of vitamin D₃ by the skin (Spina et al., 2005). Exposure of the skin to sunlight which has passed through glass windows or plastic does not result in vitamin D₃ synthesis (Holick, 1994). Sunscreens with a sun protection factor of 30 absorbs approximately 95-98% of UVB radiation, hence reducing skin vitamin D₃ synthetic capacity by 95-98% (Holick, 2007). Farmers who always wore sunscreen during summer for more than a year whilst outdoors, were reported to have low circulating 25(OH)D concentrations compared to controls (Matsuoka, Wortsman, Hanifan, & Holick, 1988). Skin thickness decreases linearly with age in humans after the age of 20 years. Therefore the skin of an elderly person will only produce 25% of the vitamin D₃ compared to

the amount a younger person will synthesise (MacLaughlin & Holick, 1985). Populations at risk of vitamin D deficiency are discussed in section 1.15.

1.7.3 Dietary absorption of vitamin D

The absorption mechanism discussed herein is for non-hydroxylated forms of vitamin D (vitamin D₂ and D₃), as no data is available yet for the hydroxylated (25(OH)D₃) forms. Vitamin D as well as other fat soluble vitamins have similar absorption in the human upper gastrointestinal tract (GIT), including emulsification, solubilisation in mixed micelles, diffusion across the unstirred water layer and its penetration through the enterocytes to the circulation (Tso & Fujimoto, 1991). The specific site of vitamin D absorption in humans is not yet known, however, in rats the ileum has been reported to be the main site (Reboul et al., 2011). After ingesting vitamin D from the diet, it is released from the food/supplement matrix in which it is embedded. To aid digestion, vitamin D is emulsified into smaller emulsion droplets by bile salts and phospholipids without any chemical modification (Reboul et al., 2006). This emulsification enables digestive enzymes including pancreatic lipase to digest vitamin D (Reboul et al., 2006). Following digestion, vitamin D₂ or D₃ is solubilised in mixed micelles by bile salts and phospholipids, and diffuse into the enterocytes where they are absorbed (Borel, Caillaud, & Cano, 2015; Reboul et al., 2006). Once inside the enterocyte vitamin D is incorporated into chylomicrons and first enters the lymphatic system then the blood stream before it enters the liver. The more vitamin D is incorporated into chylomicrons, the higher the rate of absorption or bioaccessibility (Borel et al., 2015; Reboul et al., 2006). Following this, they are absorbed through the lymphatic route into the systemic circulation, whilst 25(OH)D₃ undergoes absorption via the portal route (Borel et al., 2015; Reboul, 2015). The absorption of 25(OH)D₃ is about three times higher compared to vitamin D₂ & D₃, as it is more polar and does not depend on bile acids for absorption (Compston, Merrett, Hammett, & Magill, 1981; Maislos, Silver, & Fainaru, 1981). An *in vivo* study in rats demonstrated that lack of biliary salts did not affect absorption of 25(OH)D₃ but did reduce and impede the absorption of vitamin D₂ and D₃ by (Maislos & Shany, 1987). The absorption of 25(OH)D₃ is assumed to be a non saturable passive diffusion process. It is important to note that, intestinal cell membrane proteins such as scavenger receptor class B type 1 (SR-B1), cluster determinant 36 (CD36) and Niemann- Pick C1 like 1 (NPC1L1) are also involved in the apical absorption of

cholesterol and other fat soluble vitamins. It has been discovered that at low dietary vitamin D concentrations, absorption is protein mediated but at high dietary vitamin D concentration, absorption is passive (Reboul et al., 2011).

The vehicle for vitamin D transport is significant particularly in people with fat malabsorption problems (Grossmann & Tangpricha, 2010; Tangpricha et al., 2012). Thus, to maximise absorption from vitamin D supplementation, there is need to know the most efficient vehicle for its transport. As a result, the vehicle in which vitamin D is carried is significant in its absorption, however, available evidence is conflicting. Vitamin D in an oil vehicle has been reported to be more bioavailable than in a powder based vehicle (cellulose or lactose) or in ethanol (Grossmann & Tangpricha, 2010). However, another clinical trial discovered vitamin D to be more bioavailable from a lactose capsule compared to an oily drop formulation (Coelho et al., 2010).

Vitamin D₂ and D₃ have been reported to have equal intestinal absorption. In healthy subjects both receiving 1000 IU (25µg) of D₂ and D₃, serum 25(OH)D increased equally in all the supplemented group (Holick et al., 2008). Another study with D₂ and D₃ fortified orange juice or in a supplement was shown to give similar serum 25(OH)D₃ responses suggesting similar bioavailability (Biancuzzo et al., 2010). However, Romagnoli et al.,(2008), found a higher bioavailability of D₃ than D₂ after administering same large doses of both forms to elderly subjects. The possible reason given for the difference in bioavailability has been attributed to different affinities of D₂ and D₃ for the VDR (Borel et al., 2015).

1.7.3.1 Effectors of dietary vitamin D absorption

The components of a meal (particularly lipids), or gut enzymes secreted after a meal have been suggested to improve vitamin D absorption (Mulligan & Licata, 2010). Lipids improve the absorption of fat soluble micro constituents (FSM) by stimulating its release from the food matrix and providing a hydrophobic phase where FSM could be solubilized. By stimulating biliary secretion and micelle synthesis and by inducing chylomicron synthesis which in turn enhances FSM transport outside the enterocytes, thus, increasing FSM absorption (Borel et al., 2015). The rate of absorption of fat soluble vitamins can be improved by nanoparticles and compounds capable of forming micelles. The water-soluble form of vitamin E (Tocopheryl

succinate polyethylene glycol 1000 TPGS) has been employed to enhance vitamin D absorption, as it is capable of solubilising lipophilic compounds. A clinical study in children aged 5-19 years with severe cholestasis, found higher absorption of vitamin D administered in a mixture with TPGS (Argao, Heubi, Hollis, & Tsang, 1992).

The vitamin D status of the host is significant in its intestinal absorption, although there is limited available data on this. The intestinal absorption and hydroxylation in the liver of vitamin D₃ in rats were found to be higher in vitamin D deficient rats compared to the non-deficient rats (Apukhovskaia, Khrestovaia, Antonenko, Omel'chenko, & Dotsenko, 1990). From this finding, it could be deduced that intestinal absorption of vitamin D is higher in vitamin D deficient individuals.

1.7.3.2 Inhibitors of dietary vitamin D absorption

Several lipid lowering drugs such as tetrahydrolipstatin (orlistat) and olestra reduce the absorption of cholesterol, triacylglycerols and vitamin D, since vitamin D and lipids have similar fate in the GIT (McDuffie, Calis, Booth, Uwaifo, & Yanovski, 2002). Phytosterols may also reduce vitamin D absorption by impairing its solubilisation in mixed micelles, a finding supported by an *in vitro* study in mice showed that phytosterols compete with vitamin D for incorporation into mixed micelles as well as for apical uptake (Goncalves et al., 2011). A study by Reboul et al., (2011), found high concentrations of vitamin E to significantly disrupt vitamin D absorption. The interactions observed between vitamin D and other fat-soluble vitamins could be attributed to them sharing a similar mechanism of absorption.

Genetic factors may be significant in vitamin D absorption, as protein expression and activity are regulated by genes involved in the cellular uptake of vitamin D, although there are no available data on this (Ahn et al., 2010; Karohl et al., 2010). Genetic variations resulting in functional modifications in the amino acid sequence of proteins will simultaneously diminish the ability of these proteins to correctly perform their function in vitamin D metabolism (Borel, 2012; Lindqvist, Sharvill, Sharvill, & Andersson, 2007). Diseases including obstructive jaundice, cystic fibrosis and coeliac disease which are characterised by impaired fat absorption may obstruct vitamin D absorption. A study by Farraye et al., (2011) showed cystic fibrosis patients absorbed less than one third or one half (Lark et al., 2001) the quantity of oral vitamin D₂ that was absorbed by healthy patients. The detrimental effect of these

diseases on vitamin D bioavailability can be improved by administering hydroxylated forms of vitamin D as patients with intestinal fat malabsorption syndrome had improved absorption of 25(OH)D₃, which is absorbed directly through the portal vein (Leichtmann, Bengoa, Bolt, & Sitrin, 1991) or can be, to some extent corrected by sun exposure (Robberecht, Vandewalle, Wehlou, Kaufman, & De Schepper, 2011). A study by Aarts et al., (2011) on studying the effect of gastric surgery on vitamin D bioavailability found peak serum vitamin D₃ concentrations to be 30% lower after Roux-en-Y gastric bypass than before surgery.

Aging has not been found to be associated with vitamin D absorption. A study by (Harris & Dawson-Hughes) in 2002 of 25 young men aged 18-35 years and 25 older men aged 62-79 years who were administered a daily dose of 800 IU (20µg) of vitamin D₃ did not find a significant effect of aging on vitamin D absorption. The low vitamin D status in older people may be due to reduced dietary intake and the reduced capacity of skin to synthesise vitamin D/reduced hydroxylation by the liver and little exposure to sunlight (Borel et al., 2015).

1.8 REGULATION OF VITAMIN D METABOLISM

Renal regulation of vitamin D is influenced by four parameters: direct negative feedback by vitamin D, parathyroid hormone (PTH), calcitonin and fibroblast growth factor 23 (FGF-23).

1.8.1 Regulation by calcium/PTH

Increased PTH due to hypocalcemia is the principal signal that results in the activation of vitamin D synthesis in the kidneys (Bikle, 2014; Henry, 2011). The upregulation of CYP27B1 transcription which leads to calcitriol synthesis following raised PTH is mediated by the nuclear orphan receptor 4A2 (NR4A2) (Zierold, Nehring, & DeLuca, 2007). Vitamin D in turn directly inhibits the production of PTH in the parathyroid gland at the level of human PTH transcription promoter (Koszewski, Alimov, Park-Sarge, & Malluche, 2004) and indirectly by raising serum calcium concentrations and by activating calcium sensing receptor expression and transcription (Canaff & Hendy, 2002).

1.8.2 Feedback regulation by vitamin D

Vitamin D regulates its own production by forming a complex with VDR, this represses CYP27B1 expression (Henry, 2011), decreases PTH release and stimulates its own breakdown to water soluble compounds that are excreted through bile by triggering the production of CYP24A1 (Holick, 2011; Norman, 2008).

1.8.3 Regulation by phosphate/FGF23

FGF-23 is a 32 kDa glycoprotein expressed primarily in osteoblasts and osteocytes, belonging to the F19 subfamily and it is different from other FGFs, as it acts in an endocrine pattern (Hu, Shiizaki, Kuro-o, & Moe, 2013; Quarles, 2012). FGF23 is synthesised in response to increased phosphate concentrations, as it stimulates renal phosphate excretion in the urine by decreasing its reabsorption in the proximal renal tubules of the kidney (Liu et al., 2006; Segawa et al., 2007). Alpha Klotho (α klotho), a 130 kDa transmembrane protein expressed in the distal renal tubules of the kidneys acts as a obligate co-receptor for FGF23, α Klotho forms complexes with FGFR1c, FGFR3c and FGFR4 which enhances the FGF23 activation of FGFRs and as a result FGF23 together with α Klotho suppresses vitamin D synthesis by downregulating CYP27B1 whilst upregulating CYP24A1 involved in vitamin D catabolism (Bergwitz & Juppner, 2010). Deficiency in FGF23 or α Klotho leads to increased vitamin D synthesis, premature aging and ectopic calcification (Shimada, Kakitani, et al., 2004). FGF-23 is increased in chronic kidney disease (CKD), this leads to suppression of vitamin D and increased PTH synthesis. The increase in FGF-23, has been suggested to be an early biomarker of CKD, as FGF-23 increases due to loss of functional renal mass and impaired calcium-phosphate homeostasis (Quarles, 2012).

1.8.4 Regulation by calcitonin

In addition to decreasing blood calcium by downregulating osteoclasts under high blood calcium concentrations, calcitonin also triggers renal CYP27B1 expression which leads to increases in plasma 25(OH)D concentration under normal calcium conditions (Shinki, Ueno, DeLuca, & Suda, 1999). There is evidence that calcitonin upregulates CYP27B1 transcription in the cells of the kidneys (Zhong, Armbricht, & Christakos, 2009).

1.9 PROPOSED MECHANISM OF ACTION OF VITAMIN D

1.9.1 VDR

The VDR is responsible for mediating the biological (genomic and non-genomic) actions of vitamin D and its analogues. VDR belongs to the nuclear receptor superfamily which includes adrenal steroids, retinoic acid, thyroid and sex hormones (Margolis & Christakos, 2010). The VDR is distinct from other members of the nuclear receptor superfamily as it is coded by only one gene (Carlberg & Molnár, 2012) located on chromosome 12 and consist of 8 coding exons (Zella, Kim, Shevde, & Pike, 2006), 6 non-coding exons and at least 2 promoters (Zella et al., 2010). Human VDR protein contains 427 amino acids which heterodimerise with retinoid X receptor (RXR) for the stimulation of vitamin D target genes (Pike & Meyer, 2010; Zella et al., 2006).

The NH₂-terminal DNA binding domain (DBD) and COOH-terminal ligand binding domain (LBD) are the two principal functional domains of the VDR. DBD contains a cysteine- rich zinc finger region and there are 2 zinc fingers each of which consists of a single zinc atom in a tetrahedral arrangement with 4 invariant cysteine residues (DeLuca, 2008; Pike & Meyer, 2010). LBD contains a minimum of 12 α helices (H1-H12) (Rochel, Wurtz, Mitschler, Klaholz, & Moras, 2000). Vitamin D binding to LBD causes a conformational change that promotes its interaction with coregulatory complexes and RXR, and this is essential for target genes transcription and the recruitment of coactivator proteins (Rochel et al., 2000; Shaffer, McDonnell, & Gewirth, 2005). The VDR gene is greatly expressed in metabolic tissues including the bone, kidneys, liver and intestine, but it is low to moderately expressed in the other approximately 250 different human tissues (Verstuyf et al., 2010).

1.9.2 Genomic mechanism of action of vitamin D

On reaching a target cell, vitamin D detaches from VDBP and binds to the VDR, which in turn is phosphorylated to regulate its genomic efficiency and potency, after binding, the VDR/vitamin D complex moves from the cytoplasm to the nucleus (Haussler, Jurutka, Mizwicki, & Norman, 2011; Smolders et al., 2009). In the nucleus, the VDR/vitamin D complex forms a heterodimer by binding with 9-cis-RXR (**Fig.1.4.**), this heterodimer improves the affinity of the VDR transcriptional

response to DNA binding and assembles cofactors which form a powerful transcriptional complex. The transcriptional complex recognises and binds with specific DNA sequences called vitamin D response element (VDRE) (**Fig.1.4.**) at the promoter region of approximately 3,000 vitamin D target genes in the human genome (Bouillon et al., 2008; Christakos, Dhawan, Liu, Peng, & Porta, 2003; DeLuca, 2008; Haussler et al., 2011). Following the binding of vitamin D/VDR/RXR heterodimer to VDRE, changes in gene expression are either facilitated or repressed due to the ability of the liganded receptor to recruit transcriptional co-activators or co-repressors, resulting in the induction or suppression of protein synthesis (Haussler et al., 2011). It is important to note that VDR upregulation by vitamin D analogues such as paricalcitol will assemble a different cofactor from the ones assembled by vitamin D. This is a possible explanation for the difference observed between vitamin D and its analogues in their impact on the atherosclerotic process (Issa, Leong, Sutherland, & Eisman, 2002).

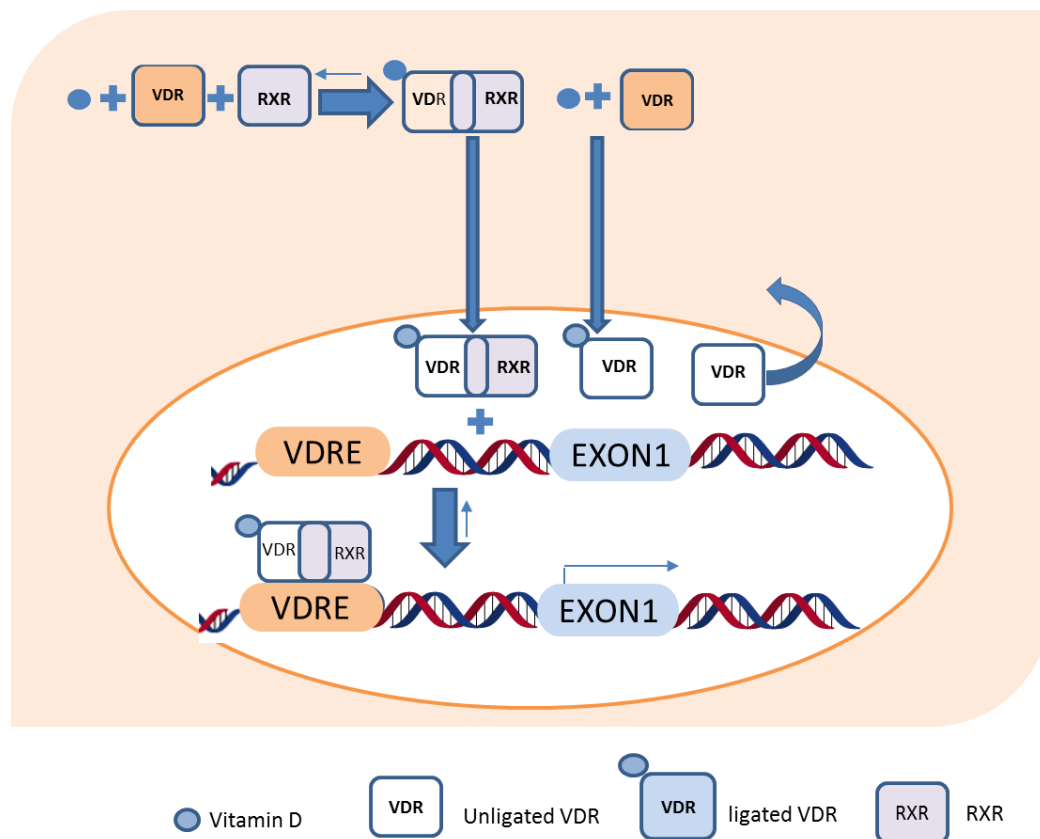


Fig.1.4. Genomic mechanism of action of vitamin D through the VDR adapted from Smolders et al.,(2009).

1.9.3 Non-genomic mechanism of action of vitamin D

Vitamin D binds to VDR associated with caveolae (cholesterol-rich invaginations present in endothelial cells and vascular smooth muscle cell (VSMC), the source of the rapid response signal transduction pathway) of the plasma membrane to stimulate non-genomic responses (Huhtakangas, Olivera, Bishop, Zanello, & Norman, 2004). This leads to the activation of rapid response signal transduction pathways including phosphatidylinositol-3'-kinase (PI3K), phospholipase C, or protein kinase C (PKC); which may open voltage-gated calcium or chloride channels (Haussler et al., 2011).

1.10 VDR GENE POLYMORPHISMS

Over 60 VDR polymorphisms located in the promoter region in and around exons 2-9 and in the 3' untranslated (UTR) region have been discovered, however, only few have been studied (McCullough, Bostick, & Mayo, 2009). Polymorphisms occur following genetic alterations of the VDR gene, this may lead to dysfunctional gene expression, affecting the endocrine/autocrine/paracrine functions of vitamin D (Valdivielso & Fernandez, 2006). The location of the VDR gene determines its function, as it differs amongst ethnic groups (Zhou, Xu, & Gu, 2009). A meta-analysis aimed at evaluating the relationship between Graves' disease (also known as toxic diffuse goiter, an autoimmune disease that affects the thyroid) and VDR gene polymorphism, found that the restriction enzymes *BsmI*, *FokI* and *Apal* polymorphisms were likely to be associated with Graves' disease in Asians but not in Caucasians (Zhou et al., 2009).

The possible role of VDR gene polymorphisms in the development of a variety of diseases has garnered attention recently, as roles in coronary artery disease (CAD) (Monraats et al., 2010) and diabetes have been reported (Rivera-Leon et al., 2015). A recent meta-analysis aimed at accurately assessing the association between VDR genetic polymorphisms and CAD risk, found *TaqI* polymorphism to be significantly associated with increase in CAD risk. They also reported increased risk in *Apal* polymorphism in CAD patients without type 2 diabetes mellitus (T2DM) and *BsmI* polymorphism in Caucasians. *FokI* polymorphism they report may possibly play a protective role in CAD (Lu et al., 2016). Individual variations occur in vitamin D synthesis, which could be due to genetic variation represented by polymorphisms of certain genes in the vitamin D metabolic pathway, especially polymorphisms of the

enzyme 7DHC reductase in the skin, 25-hydroxylase in the liver and VDBP in the circulation (Ahn et al., 2010).

1.11. PHYSIOLOGICAL FUNCTIONS OF VITAMIN D

1.11.1 Classical functions of vitamin D

The regulation of intestinal/kidney calcium absorption and maintaining calcium homeostasis are the main classical functions of vitamin D and the VDR (Christakos et al., 2016).

1.11.1.1 Intestinal calcium absorption

Vitamin D regulated intestinal calcium absorption is mediated by facilitated diffusion, through active transcellular transport of calcium across the luminal brush border membrane, cell interior and the active extrusion of calcium from the basolateral membrane into the calcium-selective channel known as Transient Receptor Potential Cation Channel Subfamily V Member 6 (TRPV6) (Peng, Brown, & Hediger, 2003). On reaching TRPV6, calcium binds with a calcium binding protein called calbindin-D_{9k}, this increases calcium release by intestinal plasma membrane ATPase (PMCA1b) across the basolateral membrane into the circulation. When dietary calcium levels are low, PMCA1b is upregulated by vitamin D (Christakos, Lieben, Masuyama, & Carmeliet, 2014). TRPV6 and calbindin-D_{9k} are the primary intestinal target of vitamin D as they are expressed in all the parts of the intestine and a strong relationship exist between their expression and efficient transcellular calcium absorption (Replogle, Li, Wang, Zhang, & Fleet, 2014; Song et al., 2003). There is evidence that rickets and osteomalacia were prevented on feeding VDR null mice a diet high in calcium and lactose. This shows that impaired intestinal calcium absorption leads to impaired bone mineralisation due to a defect in VDR signalling (Amling et al., 1999; Masuyama et al., 2003).

1.11.1.2 Renal calcium absorption

Vitamin D regulates the reabsorption of filtered calcium in the distal tubules of the kidneys through active transcellular mechanism (Riccardi & Brown, 2010). Calcium enters through the apical membrane TRPV5 and is transported to the cytoplasm by binding to calbindin-D_{9k} and calbindin-D_{28k}, following this, calcium is released by

PMCA1b and the sodium /calcium exchanger (NCX1). Reabsorption by TRPV5 is the rate limiting step in renal calcium absorption (Christakos et al., 2016). Hypercalciuria results from downregulation of TRPV5 (Hoenderop et al., 2003). Evidence shows there is reduced expression of TRPV5, calbindin-D_{9k} and calbindin-D_{28k}, NCX1 and PMCA1b in Cyp27b1 null mice which improved after vitamin D supplementation (Hoenderop et al., 2002). TRPV5 abundance at the apical membrane is regulated by FGF-23 and α Klotho through FGF-23 signalling performed by the FGFR1- α Klotho complex at the basolateral membrane, in addition to its regulation by vitamin D and PTH (Andrukhova et al., 2014).

1.11.1.3 Bone resorption

The concentration of circulating calcium in the blood depends on the movement of calcium in and out of bone, as bone morphology depends on sufficient calcium supply. Approximately 99% of body calcium is stored in the bone, and 1% is present in the extracellular and intracellular spaces (Blaine, Chonchol, & Levi, 2015). When intestinal calcium absorption is inadequate in the case of negative calcium balance, calcitriol levels increase and act directly on osteoblasts/osteocytes to shift calcium from the skeleton to blood so as to maintain normal concentrations (Christakos et al., 2014). To maintain normal calcium homeostasis, VDR signalling stimulates bone resorption indirectly by acting on osteoblast and osteoprogenitors which directly induce the transcriptional expression of RANKL, a significant osteoclastogenic factor (Kim, Yamazaki, Zella, Shevde, & Pike, 2006). RANKL then binds to its cognate receptor RANK in osteoclast precursors stimulating osteoclast formation and action as well as osteoprotegerin (OPG), a decoy receptor of RANKL (Christakos et al., 2016), thereby decreasing bone mass and inhibiting bone matrix mineralisation but maintaining sufficient serum calcium concentrations (Lieben & Carmeliet, 2013).

1.11.1.4 Maintenance of calcium homeostasis

The maintenance of calcium homeostasis is crucial for bone mineralisation and neuro-muscular functions (**Fig.1.5**) (DeLuca, 2008; Holick, 2011). Calcium concentration is tightly regulated at approximately 1mmol/L and 2.5mmol/L for ionised and total calcium respectively (Blaine et al., 2015). PTH, calcitonin and vitamin D together regulate calcium homeostasis, with PTH being the most significant regulator (Blaine et al., 2015). As serum calcium concentrations fall

below normal, calcium sensing proteins in the parathyroid gland (PTG) stimulate PTH secretion into the circulation within seconds in response and PTH raises calcium concentration by stimulating calcium release from bone, by stimulating bone resorption. PTH decreases urinary loss of calcium by stimulating calcium reabsorption and finally stimulates intestinal calcium absorption by inducing synthesis of vitamin D in the kidney. The PTG cascade of events stops as calcium concentration increase (Blaine et al., 2015), but when serum calcium concentration surpasses the set point of the calcium sensing system, the thyroid gland is stimulated to produce calcitonin. Calcitonin lowers serum calcium concentration by obstructing calcium mobilisation from bone to restore homeostasis (Zierold, Mings, & DeLuca, 2003).

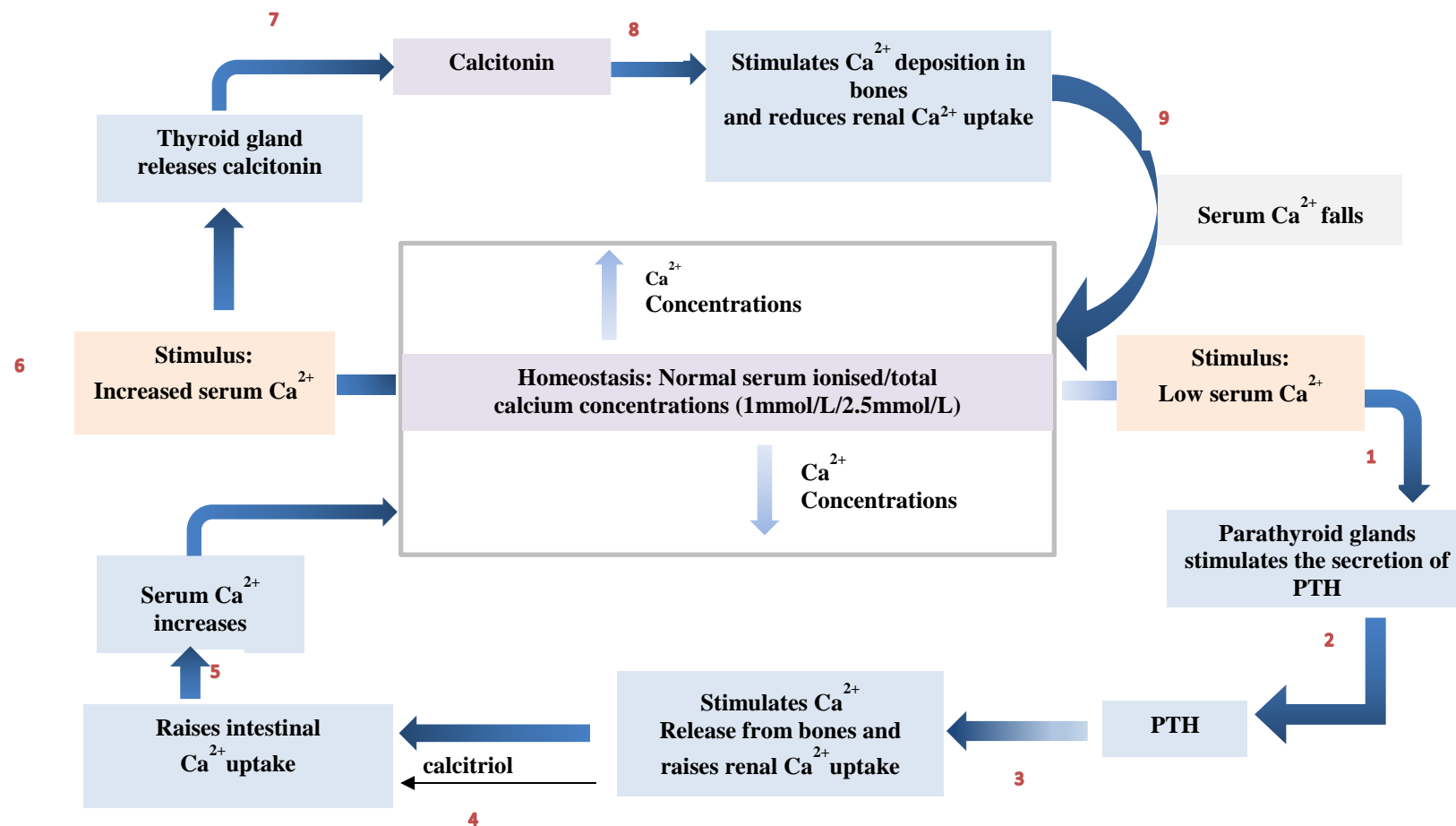


Fig.1.5. The maintenance of calcium homeostasis

1.12 VITAMIN D STATUS

A general agreement has been reached globally that circulating 25(OH)D concentrations <25 nmol/L (10 ng/mL) is regarded as vitamin D deficiency. However, no agreement has been reached on standard definition of 'optimal' vitamin D (Cancer Research UK, 2010; SACN, 2007), as for some organs and tissues, vitamin D acts as a threshold nutrient (Heaney, 2008). The absence of an agreement and definition between countries has led to the use of associated values to describe vitamin D status, including the terms 'deficiency', 'insufficiency', 'adequacy' and 'optimal'. Due to difference in the cut-off levels used together with the interchangeable terminologies, it is difficult to make correct comparisons of reported prevalence (Hilger et al., 2014; Thacher & Clarke, 2011). A 25(OH)D concentration of <25 nmol/L is used as the cut-off value defining vitamin D deficiency in the UK (SACN, 2016). This cut-off value was set with regards to preventing bone diseases including rickets and/or symptomatic osteomalacia.

The US Institute of Medicine (IOM), defined deficiency as serum 25(OH)D concentration <30 nmol/L, serum 25(OH)D concentration of 30–50 nmol/L as insufficient in some people and serum 25(OH)D concentration >50 nmol/L as sufficient for 97.5% of the population (Ross et al., 2011). This report however, does not support the proposal that all adults should have plasma concentrations of 25(OH)D >75 nmol/L. The UK National Osteoporosis Society, suggested the IOM vitamin D thresholds be adopted by UK practitioners (National Osteoporosis Society, 2013). The Endocrine Society Task Force does not support the IOM recommendations and thus published a clinical practice guideline that defined deficiency as serum 25(OH)D <50 nmol/L. The guideline recommended that 25(OH)D concentration should be >75 nmol/L to exert the beneficial effects of vitamin D on calcium, bone and muscle metabolism (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, Weaver, et al., 2011). Additionally, the World Health Organisation (WHO) also defined deficiency and insufficiency as serum 25(OH)D <25 nmol/L and 50 nmol/L respectively (Lamberg-Allardt, Brustad, Meyer, & Steingrimsdottir, 2013). Based on a study investigating the effect of vitamin D intervention on bone quality in the elderly (Weggemans, Schaafsma, & Kromhout, 2009), the Health Council of the Netherlands, set at least 50 nmol/L for

optimal serum 25(OH)D level all year round for women aged 50 and over, and for men aged 70 and over (Health Council of the Netherlands, 2012).

Based on critical discussions in the consensus for optimal serum 25(OH)D concentrations from Central Europe, including Poland, Hungary, Belarus, Estonia, Czech Republic and Ukraine (Pludowski et al., 2013). Serum 25(OH)D concentrations were established as <50 nmol/L, 50-75 nmol/L and 75-125 nmol/L for deficiency, insufficiency and optimal respectively. However, evidence to support the requirement to aim for significantly higher serum 25(OH)D concentrations of >75 nmol/L is unclear (Bouillon et al., 2013). The basis for optimal vitamin D status has moved away from being defined as the 25(OH)D required for skeletal functions to that which will portray maximum benefits on non-skeletal functions (Thacher & Clarke, 2011). (Garland, French, Baggerly, & Heaney) in 2011, suggested that serum 25(OH)D concentration of 150-200nmol/L (60-80ng/mL) was required to reduce cancer risk. In 2009, (Ginde, Mansbach, & Camargo) discovered that the protective effect of vitamin D from upper respiratory infections is a serum 25(OH)D concentration >75nmol/L (30ng/mL). The optimal vitamin D concentration for reducing insulin resistance has been shown to be 80-119nmol/L (Takiishi, Gysemans, Bouillon, & Mathieu, 2010).

The difficulty in defining the exact thresholds of 25(OH)D concentrations may be due to difficulty in comparing results across studies due to assay variations of 25(OH)D concentrations between different laboratories/a lack of standardisation of methodology. To amend this, the International Vitamin D External Quality Assessment Scheme (DEQAS) was created in 1989 to monitor the accuracy of individual laboratories. Laboratories which participate get a certificate when 80% of their results from the five quarterly samples issued by (DEQAS) are within 30% of the DEQAS All - Laboratory Trimmed Mean (ALTM) (Carter et al., 2010). A pass mark by DEQAS simply means the method is relatively correct (Carter, 2009). According to the first vitamin D standardisation certification program administered by the US Centers for Disease Control and Prevention, annual certification of laboratories is obtained when test results have a bias of $\pm 5\%$ and a reproducibility of $\leq 10\%$ (Glendenning, 2015). The standardisation of methodology is critical to conclude on reliable thresholds for deficiency and insufficiency and to identify poor 1,25(OH)₂D₃ status effectively (Spiro & Buttriss, 2014).

1.12.1 Vitamin D toxicity

Vitamin D toxicity is characterised by hypercalcaemia and hyperphosphataemia, which results in deposition of calcium in soft tissues. Vitamin D toxicity occurs by very high and inappropriate high dose treatment or overdosing (Hathcock, Shao, Vieth, & Heaney, 2007), however, a daily dose of 10,000IU (250µg) is reported as safe (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003), as food supplements containing up to 10,000 IU (250µg) of vitamin D per daily dose are available in the UK (SACN, 2016). However, doses equal to or above 50,000IU/day for several weeks or months are associated with toxicity and hypercalcaemia. Toxicity, defined by hypercalcaemia has not been observed with a serum 25(OH)D concentration < 500nmol/L (National Osteoporosis Society, 2013).

1.13 RECOMMENDATIONS FOR VITAMIN D INTAKE

The Dietary reference values/recommendations for vitamin D from national authorities differ among countries (Prentice, 2002). The Scientific Advisory Committee on Nutrition on Vitamin D and health published on 21st July 2016 proposed an RNI in the UK population. A dose of 400 IU (10µg) daily was proposed from ages four and above, including at risk groups, 8.5 - 10µg daily for all infants from birth to one year including exclusively breast fed infants and 10µg daily for children aged one to four years (Table 1.3). The chosen dose is the average amount required to maintain a serum 25(OH)D concentration > 25nmol/L and to protect musculoskeletal health (SACN, 2016).

Table1.3. Reference Nutrient Intake for vitamin D in the UK (µg/d) (SACN, 2016)

Age	RNI (µg/day)
0-12 months	8.5-10µg/day
12 months - 4 years	10 µg/day
4years and above	10µg/day
Populations at risk of deficiency	10µg/day

The United States Endocrine Society's Clinical Practice Guideline recommends 600-1000 IU/d (15-25µg) for children aged one or more, and 1500-2000 IU/d (37.5-50 µg) for adults aged 19 years and above (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011). This is aimed at maintaining 25(OH)D above the optimal level of 75nmol/L. The IOM recommended 400 IU (10 µg/d) for infants between 0-12 months, 600 IU (15µg) for people between age 1 – 70 years, including pregnant and lactating women and 800 IU for people > 70 years (Ross et al., 2011). The predictive regression equation study for vitamin D replacement suggested that a daily dose of 5,000 IU (125µg) vitamin D would be required to effect a 25nmol/L increase in 25(OH)D, as they discovered each IU of vitamin D results in a 0.005nmol/L ($10/0.002 = 5000$) increase. The vitamin D dose $\geq 2,000$ IU (50µg) was recommended for maintenance (Singh & Bonham, 2014). The findings of this analysis are similar to the recommendations made by the Endocrine Society. The Endocrine Society suggested that in obese and malabsorption syndromes patients, patients on medications inhibiting vitamin D metabolism, a daily dose of at least 6,000 IU (150µg) to 10,000 IU (250µg) of vitamin D is required to treat vitamin D deficiency and to maintain 25(OH)D >75nmol/L. This should be followed by a daily maintenance therapy of 3000 (75µg) to 6000 IU (150µg) once vitamin D deficiency has been improved (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, Weaver, et al., 2011).

1.14 PREVALENCE OF VITAMIN D DEFICIENCY IN THE UK

Data from the National Diet and Nutrition Survey, the Health Survey for England, the Low Income Diet and Nutrition Survey, the UK Diet and Nutrition Survey of Infants and Young Children and the Scottish Health Survey were used to assess the vitamin D status of the UK population (SACN, 2016). Results from these surveys in UK show that around a fifth of adults aged 19 to 64 years and adults aged 65 years and over and a sixth of children aged 11 to 18 years had 25(OH)D concentrations < 25nmol/L (Bates et al., 2014). The prevalence of vitamin D deficiency in the UK by sex and age groups in infants, young children and the rest of the UK population are shown in **Fig.1.6 and 1.7**.

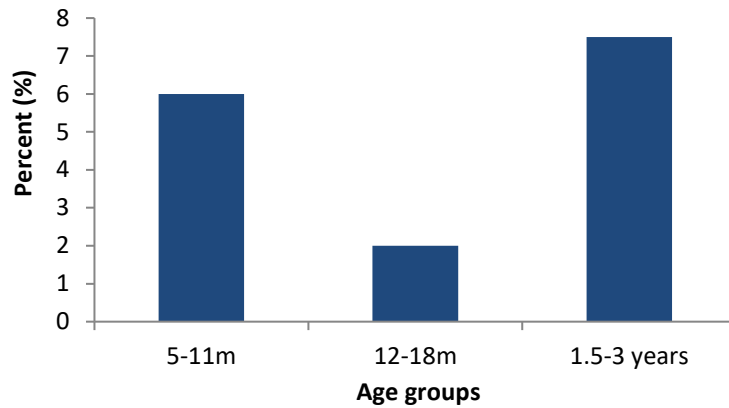


Fig.1.6. Prevalence of vitamin D deficiency in infants and young children in the UK (SACN, 2016)

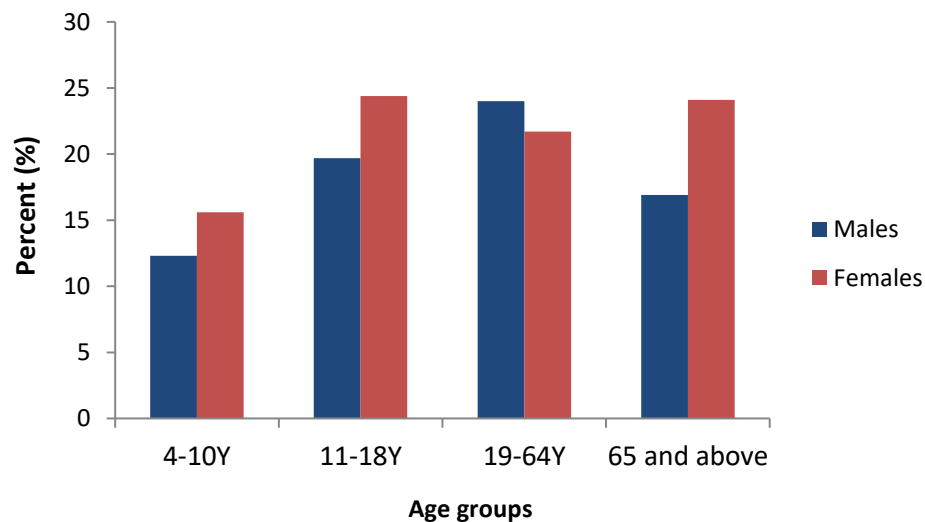


Fig.1.7. Prevalence of vitamin D deficiency in the UK by sex and age groups (SACN, 2016)

Several sub-populations of young and older adults have shown particularly low vitamin D concentrations within the UK (Lawson & Thomas, 1999), particularly in children, adolescents and women of south Asian and African Caribbean origin. It has been reported that $\geq 50\%$ of pregnant women from ethnic minorities in UK, 18% of pregnant women in southern England had a serum 25(OH)D concentration $\leq 25\text{nmol/L}$ and 31% had a 25(OH)D concentration $\leq 50\text{nmol/L}$ indicating a high

prevalence in Whites (Datta et al., 2002). Low dietary intake of vitamin D in the UK has been implicated as the cause (Javaid et al., 2006). Regional differences of 25(OH)D concentrations have been observed in the UK. A nationwide cohort study by Hypponen and Power (2007), found women of European ancestry, particularly those living in Scotland to have a higher prevalence of 25(OH)D \leq 25nmol/L, than those living in the South of England. A longitudinal study in young South Asian women living in the UK reported that serum 25(OH)D concentrations \leq 25 nmol/L were highly prevalent in South Asian women of child bearing age during winter (81%) and autumn (79.2%) (Darling et al., 2013).

1.15 POPULATIONS AT RISK FOR VITAMIN D DEFICIENCY

Several populations have been identified to be at risk of vitamin D deficiency and they include:

1.15.1 Infants

Infancy is characterised by a high rate of skeletal growth/ossification and with tooth enamel formation, as a consequence, infants are more vulnerable to vitamin D deficiency (Harvey, Dennison, & Cooper, 2014). Exclusively breast-fed infants are particularly at risk because of decreased concentrations of vitamin D and its metabolites in human breast milk which provides <25IU/L (0.62 μ g) to 78IU/L (1.99 μ g). An infant's vitamin D status is determined by the mother's vitamin D status during pregnancy (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011). Hollis and Wagner (2004), reported that a dose of 400 IU (10 μ g/d) vitamin D supplementation during lactation is insufficient to raise breast milk vitamin D and infant 25(OH)D concentrations and thus recommended a higher dose.

1.15.2 Older people

Age can negatively affect the concentrations of previtamin D₃ as the proportion of 7DHC available in skin decreases with age (Webb & Engelsen, 2006). Older adults, particularly those living in institutions are at higher risk of deficiency due to age related decline in dermal synthesis and decline in the rate of renal hydroxylation which results in the active hormonal form. Limited skin exposure as well as target tissue response as observed in bone, also affects the concentration of previtamin D₃

(Shearer, 1997). Under equal exposure to sunlight a 70 year old will produce 75% less vitamin D than a 20 year old (Hagenau et al., 2009). Studies in rats have shown that renal CYP24A1 increases with increasing age (Armbrecht, Zenser, & Davis, 1980; Matkovits & Christakos, 1995).

1.15.3 People with dark skin

People with black skin have lower serum 25(OH)D concentrations in winter and summer compared to Whites (Harris & Dawson-Hughes, 1998). This is because the pigment melanin in dark skin has an absorption spectrum of 290-700 nm and thus effectively absorbs UVB radiation which reduces the skin's ability to synthesise vitamin D from sunlight (Jablonski & Chaplin, 2000). Thus, people with darker skin require a longer period of UVB exposure to produce an equivalent amount of vitamin D than a fairer skinned person (Chen et al., 2007). Evidence has shown that 4% of white and 42% of black women aged 15 to 49 years had serum 25(OH)D concentrations < 37.5 nmol/L in the National Health and Nutrition Examination Survey (NHANES) from the US. This equates to 20 times higher risk for Blacks than for non-Hispanic whites (Nesby-O'Dell et al., 2002). Another reason for lower serum 25(OH)D concentrations may be ascribed to the lower concentrations of VDBP in blacks compared to whites (Schwalfenberg & Genuis, 2010). The lower 25(OH)D concentrations observed could be attributed to the high prevalence of VDBP genetic polymorphisms (rs7041 and rs4588), as Blacks have a greater chance than whites of having the T allele at rs7041. The T allele at rs7041 is associated with decreased concentrations of VDBP, however, this low VDBP may protect against the adverse effects of vitamin D deficiency (Powe et al., 2013).

1.15.4 Fat and intestinal malabsorption syndrome.

As previously mentioned in section 1.7.3.1, vitamin D being fat soluble, requires some dietary fat in the gut for absorption, thus people with reduced tendency to absorb dietary fat including people with cystic fibrosis, coeliac, inflammatory bowel disease, such as Crohn's and ulcerative colitis and gastric bypass individuals will require supplementation (Adams & Hewison, 2010). Individuals who suffer from severe malabsorption syndromes are more likely to be vitamin D deficient because the small intestine cannot absorb this vitamin (Holick, 2006). Individuals taking plasma cholesterol reducing medications such as bile acid-binding polymers

including colestyramine and colestipol would also have impaired vitamin D absorption (Holick, 2007; Tsiaras & Weinstock, 2011).

1.15.5 Overweight /Obese individuals

Obese/overweight (defined as a BMI ≥ 30 and BMI of 25-29.9kg/m² respectively) (Caballero, 2007) individuals compared with normal weight individuals (BMI between 18.5 -24.9kg/m²) have a high prevalence of serum 25(OH)D concentration < 50 nmol/L (Forrest & Stuhldreher, 2011). Studies have shown associations between 25(OH)D concentrations and obesity (Blum et al., 2008; Drincic, Armas, Van Diest, & Heaney, 2012; Vimalleswaran et al., 2013). The study by Blum et al. (2008), found lower serum concentration of vitamins D₃ and D₂ in 50% of obese compared with non-obese individuals. The increased risk of low serum 25(OH)D concentrations in overweight and obese individuals was attributed to increase in metabolic clearance of vitamin D through enhanced sequestering by adipose tissue. This may be due to the dilution of ingested or cutaneously synthesised vitamin D in the enlarged fat mass (Drincic et al., 2012). A bi-directional genetic study that adjusted for confounding concluded that a higher BMI leads to reduced 25(OH)D concentration, as each unit increase in BMI was found to be associated with 1.15% reduced serum 25(OH)D concentrations (Vimalleswaran et al., 2013).

Additionally, there is reduced rate of 25(OH)D synthesis due to hepatic steatosis (Targher et al., 2007), and the suppression of 25(OH)D synthesis by adipocytokines, macrophage migration inhibitory factor (MIF) and interleukin 6 (IL-6) secreted by adipose tissue (Ding, Parameswaran, Blizzard, Burgess, & Jones, 2010). Non-genomic mechanisms including protein expression, oxidative stress, inflammation and cellular metabolism also link vitamin D to obesity (vinh quoc Lu'o'ng & Nguyen, 2013). Overweight/obese individuals are likely to be physically inactive, and spend more time indoors, hence reducing their exposure to sunlight. A study performed in Australia reported that the efficacy of vitamin D supplementation depends on BMI, as overweight and obese people who are vitamin D insufficient may require higher doses of vitamin D to achieve vitamin D repletion compared with non-obese/overweight individuals (Lee, Greenfield, Seibel, Eisman, & Center, 2009). Forsythe et al. (2012), suggested that an individual's BMI be considered when

deciding sufficient winter dietary vitamin D intake for healthy adults residing at higher latitudes.

1.15.6 Pregnant women

There is evidence that vitamin D deficiency during pregnancy is linked with numerous adverse health outcomes in mothers, neonates and children (Dawodu & Akinbi, 2013; Fernandez-Alonso et al., 2012; Vandevijvere, Amsalkhir, Van Oyen, & Moreno-Reyes, 2012). The infants of vitamin D deficient mothers are at higher risk of vitamin D deficiency if they are exclusively fed breast milk for an extended period (Shaw & Mughal, 2013), compared to formula fed infants, as baby formulas are vitamin D fortified (NICE 2007; EFSA 2012). A randomised controlled trial (RCT) conducted in the USA in which pregnant women received either 400 IU (10µg), 2000 IU (50µg) or 4,000 IU(100µg/d) of vitamin D₃ until delivery, found 4000 IU/d (100µg) supplementation to be most effective in achieving vitamin D sufficiency throughout pregnancy without an increased risk of toxicity. Thus, it has been advocated that the current vitamin D estimated average requirement (EAR) and recommended daily allowance (RDA) for pregnant women be raised to a daily dose 4,000IU (100µg) of vitamin D₃. The RDA should be administered irrespective of race, for ideal nutritional and hormonal vitamin D status throughout pregnancy (Hollis, Johnson, Hulsey, Ebeling, & Wagner, 2011).

1.15.7 People living at high latitudes

Solar UVB radiation varies with latitude, time of the day and year. It is particularly lower at northern latitudes during winter, this is because mean concentrations of 25(OH)D follow a north to south gradient, therefore people living in northern latitudes tend to have lower 25(OH)D than people living in the south. From mid-October to the beginning of April at latitudes of about 52° and above (UK is at latitude 50-60° N), there is no UVB radiation of appropriate wavelength for the photosynthesis of previtamin D₃. Thus, little or no vitamin D₃ is produced in the skin during the winter for the remaining months of the year (Chapuy et al., 1997).

1.16 NON-CLASSICAL FUNCTIONS OF VITAMIN D

Vitamin D also performs non-classical functions due to the presence of the VDR in all tissues and cells of the human body (Holick, 2007). Studies have shown that vitamin D through direct and indirect mechanisms regulate more than 200 genes including cellular differentiation, proliferation, angiogenesis and apoptosis (Nagpal et al., 2005). Vitamin D has been reported to play significant roles in numerous diseases (Lai & Fang, 2013), thus, associations between low vitamin D concentration with cardiometabolic disease has been observed (Zittermann & Koerfer, 2008). Large observational studies and meta-analyses have shown that low 25(OH)D concentrations are a significant risk marker for a broad range of cardiometabolic risk factors including hypertension, dyslipidaemia, abnormal glucose homeostasis and atherosclerosis (Grandi, Breitling, & Brenner, 2010; Parker et al., 2010; Pilz et al., 2011; Pittas et al., 2010; Wang, Song, et al., 2012).

1.16.1 Vitamin D and the immune system

Due to the presence of VDR in macrophages, dendrites, T and B cells (Toubi & Shoenfeld, 2010), calcitriol is capable of regulating both adaptive and innate immune defence against bacterial infections by upregulating cathelicidins, an antimicrobial peptide found within the lysosomes of macrophages, keratinocytes, lung epithelial cells and polymorphonuclear leukocytes amongst others (Schauber et al., 2007; Segaert, 2008; Yim, Dhawan, Ragunath, Christakos, & Diamond, 2007). Cathelicidin regulates the transcription of VDR at the promoter region (Gombart, Borregaard, & Koeffler, 2005), thus, repressing the onset of many inflammatory diseases including lyme arthritis, T1DM, thyroiditis and inflammatory bowel disease amongst others (Adorini et al., 2004; Hayes, Nashold, Spach, & Pedersen, 2003). Vitamin D deficiency has been reported to be associated with the loss of immune tolerance (Kamen & Aranow, 2008). Calcitriol has been shown to be immunosuppressive as it has been associated with a decrease in IL-2, IFN γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA level by inhibiting the formation of the nuclear factor of activated T-cells/ activator protein 1 (NFATp/AP-1) complex through the VDR/RXR heterodimer (Raghuwanshi, Joshi, & Christakos, 2008). A high prevalence of vitamin D deficiency has been reported to be associated with increased disease activity in systemic lupus erythematosus (SLE) patients (Kamen et

al., 2005). An inverse association was observed between serum 25(OH)D concentration and disease activity in 198 African American and Hispanic SLE patients ($r = -0.234$, $P = 0.002$) indicating a high prevalence of vitamin D deficiency in SLE (Ben-Zvi et al., 2010). A systematic review and meta-analysis of 7 observational studies has demonstrated that patients with tuberculosis have lower circulating serum 25(OH)D concentrations compared to healthy same sex and age matched controls (Nnoaham & Clarke, 2008). This observation suggests low serum 25(OH)D concentrations may alter cell-mediated immunity resulting in tuberculosis (Nnoaham & Clarke, 2008).

1.16.2. Vitamin D and cancer

Calcitriol plays protective roles in prostate, breast and colon cancer (Zittermann, 2003), and may be useful in the treatment of leukaemia and other myeloproliferative disorders, as it inhibits the proliferation of some human leukaemia cell lines and promotes the differentiation of normal and leukaemic myeloid precursor. This increases the maturation and reduces the potency of potential leukemic cells (Abe et al., 1981). Calcitriol exerts a protective role against cancer cells by regulating cell maturation, differentiation and apoptosis (Zittermann, 2003). This inhibits the expression of anti-apoptotic proteins such as *Bcl2* in cancer cells and the arrest of cell cycle in G0/G1, which in turn reduces the rate of proliferation (Welsh, 2007). Calcitriol also exerts a protective role by promoting the transcription of cyclin-dependent kinase inhibitor p21 (regulator of cell cycle progression), which inhibits the growth and stimulate the differentiation of monocyte-macrophage cell lineage (Liu, Lee, Cohen, Bommakanti, & Freedman, 1996). Calcitriol promotes the synthesis of the cyclin-dependent kinase inhibitor p27 (Li et al., 2004) and suppresses the TGF- α /EGFR growth pathway. The over-expression of this pathway leads to the proliferation of tumour cells (Cordero et al., 2002). Additionally, calcitriol triggers the expression of C/EBP β , which alters the carcinogenic cell cycle protein D1 (Lamb et al., 2003) and prevents the proliferation of C/EBP β isoform LIP which enhances the activity of carcinogenic cyclin D1 (Zahnow, 2002).

Breast cancer is a common disease and a leading cause of death in women (Khodarahmi & Azadbakht, 2014), and calcitriol exerts vital roles in the mammary gland by modulating calcium transport during lactation, differentiation of hormones,

and milk protein production (Khan et al., 2014). There is evidence that calcitriol reduces benign epithelial proliferation disorders and subsequently the breast cancer risk by decreasing breast mammographic density as well as its antiproliferative and prodifferentiation activities (Chen et al., 2010). It has been reported that increased serum 25(OH)D concentrations is associated with lower breast cancer risk (Chlebowski, 2011). A review of 37 articles aimed at investigating the relationship between breast cancer and vitamin D synthesised by skin, by diet or supplementation found an inverse association between plasma 25(OH)D concentration and breast cancer risk (Stoll, Akladios, & Mathelin, 2013). Additionally, a meta-analysis of 5 prospective cohort studies with a follow-up of 2.7–24 years among breast cancer patients also demonstrated that increased serum 25(OH)D concentrations had a significant reduced risk of overall mortality (hazard ratio [HR] = 0.62, 95% CI = 0.49–0.78) and breast cancer-specific mortality (HR = 0.57, 95% CI = 0.38–0.84) compared to patients with low 25(OH)D serum concentrations (Maalmi, Ordonez-Mena, Schottker, & Brenner, 2014).

1.16.3 Vitamin D in diabetes mellitus, kidney and cardiometabolic disease

Calcitriol regulates blood pressure, electrolyte levels and volume status through a negative feedback on the renin-angiotensin system (RAS), thus, vitamin D deficient patients may be predisposed to hypertension or diseases associated with high plasma renin activity (Li, 2003). Knockout mice lacking VDR showed significant increase in BP, cardiac hypertrophy and water retention as a result of increased circulating renin, angiotensin II concentrations (Li et al., 2002). Paricalcitol, a vitamin D analogue has been reported to inhibit renal interstitial myofibroblast (Tan, Li, & Liu, 2006), hence, inhibiting the development of renal interstitial fibrosis (Foltyn et al., 2005) through its anti-inflammatory and anti-fibrotic effect (Tan, Wen, & Liu, 2008). Vitamin D deficiency causes disruptions in insulin secretion and the immune system, hence, its association with chronic diabetes mellitus (Lai & Fang, 2013). A study in type 1 diabetes mellitus (T1DM) patients with low vitamin D status compared to healthy age matched controls found out that 3 major genes controlling 25(OH)D₃ were implicated in T1DM incidence (Cooper et al., 2011). Evidence from *in vivo* and *in vitro* studies showed that calcitriol prevents the destruction of pancreatic β cells, reducing T1DM incidence (Zittermann & Gummert, 2010).

Calcitriol maintains cardiovascular health by binding to VDR on myocardial cells, thereby, regulating the hypertrophy of myocardial cells and both synthesis and release of atrial natriuretic peptide (Wu, Garami, Cao, Li, & Gardner, 1995; Wu, Garami, Cheng, & Gardner, 1996). Calcitriol inhibits vascular calcification by regulating the activities of interleukins (Schurgers et al., 2001) and by inhibiting angiogenesis and inflammatory cytokines such as TNF- α and increasing matrix Gla-protein synthesis (Muller et al., 1992). Calcitriol inhibits the proliferation and migration of VSMCs and reduces endothelial cell oxidative stress (Menezes, Lamb, Lavie, & DiNicolantonio, 2014).

Given the reported evidence for a beneficial role of vitamin D in diabetes and CVD pathogenesis and the prevalence of vitamin D deficiency, it is important to investigate the effects of vitamin D on selected cardiometabolic markers and to evaluate whether improving vitamin D status in a cohort with insufficient vitamin D concentrations would decrease cardiometabolic disease risk (discussed in detail in chapter 3). Additionally, due to the reported renoprotective role of vitamin D in the kidneys, it also important to investigate the prevalence of vitamin D deficiency and the association of vitamin D status with selected CVD markers in calcium oxalate kidney stones patients.

2.0 THE EFFECT OF VITAMIN D SUPPLEMENTATION AND CARDIOMETABOLIC RISK IN A COHORT OF OBESE/OVERWEIGHT ADULT MALES IN UK

- A systematic review on the effect of vitamin D supplementation on endothelial and inflammatory markers carried out as a basis for the literature review of this chapter was presented at the 19th International Vitamin D Workshop, 29th -31st March, 2016, at Boston, USA (Appendix 10).
- A research article entitled, “Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: A systematic review” was published in the Journal of Steroid Biochemistry and Molecular Biology (Agbalalah, Hughes, Freeborn, & Mushtaq).

2.0 THE EFFECT OF VITAMIN D SUPPLEMENTATION AND CARDIOMETABOLIC RISK IN A COHORT OF OBESE/OVERWEIGHT ADULT MALES IN UK

2.1 INTRODUCTION

2.1.1 CARDIOMETABOLIC DISEASE RISK

Cardiometabolic disease risk are various interrelated risk factors including insulin resistance, inflammation, abdominal obesity, dyslipidaemia, oxidative stress and hypertension that increase the risk of T2DM and atherosclerosis (Aschner, 2010; Kassi, Pervanidou, Kaltsas, & Chrousos, 2011). Atherosclerosis, reported to be responsible for majority of CVD events, is an inflammatory disease of the intima and is characterised by lipoprotein accumulation and oxidation, vascular smooth muscle cell migration, foam cell development and calcium deposition (Aschner, 2010; Hansson, 2005).

2.1.2. EPIDEMIOLOGY OF CVD AND T2DM IN THE UK

CVD is responsible for 17.3 million deaths globally (Naghavi et al., 2015), and for over 45% and 26% of all deaths in Europe and UK respectively (Townsend et al., 2016). The major cause of CVD death in the UK has been attributed to coronary heart disease (CHD), as it accounts for approximately 70,000 annual CVD deaths in the UK (Townsend et al., 2016). Stroke, another major cause of CVD death, second to CHD is responsible for 40,000 deaths in the UK (Townsend et al., 2016). Currently, it has been estimated that 7 million people live with CVD in the UK (3.5 million men and 3.5 million women) (Townsend et al., 2016).

Diabetes has been reported to affect 4.5 million people in the UK, 90% of which suffer from T2DM and over 1 million people are estimated to be currently living with undiagnosed T2DM in the UK, 56% of which are men (Townsend et al., 2016). Data from the Health Survey for England estimates that 10.7% of the English population (approximately 5 million people) are at increased risk of T2DM with an HbA1c concentration between 42-46 mmol/mol (6.0-6.4)(Public Health England, 2015).

2.1.3. PATHOGENESIS OF ATHEROSCLEROSIS

The 3 layers of the artery are intrinsic to understanding plaque formation in the endothelium. The inner layer (tunica intima) of the artery is lined by endothelial cells that limit larger molecules from crossing it (Reymond, d'Água, & Ridley, 2013). The middle layer (tunica media) of the artery consist of VSMCs embedded within an extracellular matrix consisting of collagen and elastin (Ponticos & Smith, 2014). The outermost layer (tunica externa) of the artery contains fibroblasts collagenous tissue (Libby & Hansson, 2015) (**Fig 2.1**). Atherosclerosis begins with the disruption of endothelial cell homeostasis which upsets the balance between vasoconstriction and vasodilation resulting in endothelial dysfunction (Davignon & Ganz, 2004; Harja et al., 2008). In response to endothelial dysfunction, lipoproteins particularly LDL-C accumulate in the sub-endothelial space of the damaged endothelium and become oxidised and modified by the reactive oxygen species (ROS) produced by both endothelial and VSMCs (Perrotta & Aquila, 2015). The ensuing endothelial dysfunction, oxidation and modification of LDL-C directly stimulates the inflammatory tissue cascade by promoting the infiltration of inflammatory cells, including monocytes, into the intima layer, and indirectly, through the induction of cytokines and other inflammatory mediators (**Fig 2.1**) (Davignon & Ganz, 2004; Harja et al., 2008).

On reaching the intima layer, the monocytes transform into a macrophage and engulf the LDL-C collected in the damaged endothelium. These macrophages die off whilst engulfing LDL-C to become foam cells also known as lipid laden macrophages, which in turn accumulate to form the atherosclerotic plaque (**Fig 2.1**) (Shaw, 2004). The foam cells formed, produce pro-inflammatory mediators, ROS, and tissue factor pro-coagulants that intensify local inflammation and stimulate thrombotic complications (Fuentes Q et al., 2013; Ross, 1999). The inflammation stimulated by dead macrophages in the plaque core activate T cells, which differentiate to T helper cell 1 (Th1) that secretes cytokine interferon gamma (IFN γ) that maintains the inflammatory state in the plaque (Hansson & Hermansson, 2011).

VSMCs in the inflammatory state, migrate from the media layer into the intima or fatty plaque and go on to produce extracellular matrix (ECM) components and a fibrous cap made up of collagen and elastin protein that cover the plaque (**Fig 2.1**). The VSMCs also induce calcium deposition in the plaque core which hardens or

calcifies as a result (Orr, Yurdagul Jr, & Patel, 2014). A rupture in the fibrous cap may lead to a large thrombus formation, which will in turn restrict blood flow or occlude the artery. If the thrombus occludes a cerebral artery, a stroke may result and if it occludes a coronary artery, myocardial infarction (MI) may result (**Fig 2.1**) (Orr et al., 2014; Roberts, 2013). The fibrous cap may remain intact and the plaque would continue to increase in size and may eventually obstruct the artery (ischaemic) where it is present/or which houses it. On the other hand, the media beneath the plaque may rupture and weaken the vascular wall (aneurysm) (Bentzon, Otsuka, Virmani, & Falk, 2014).

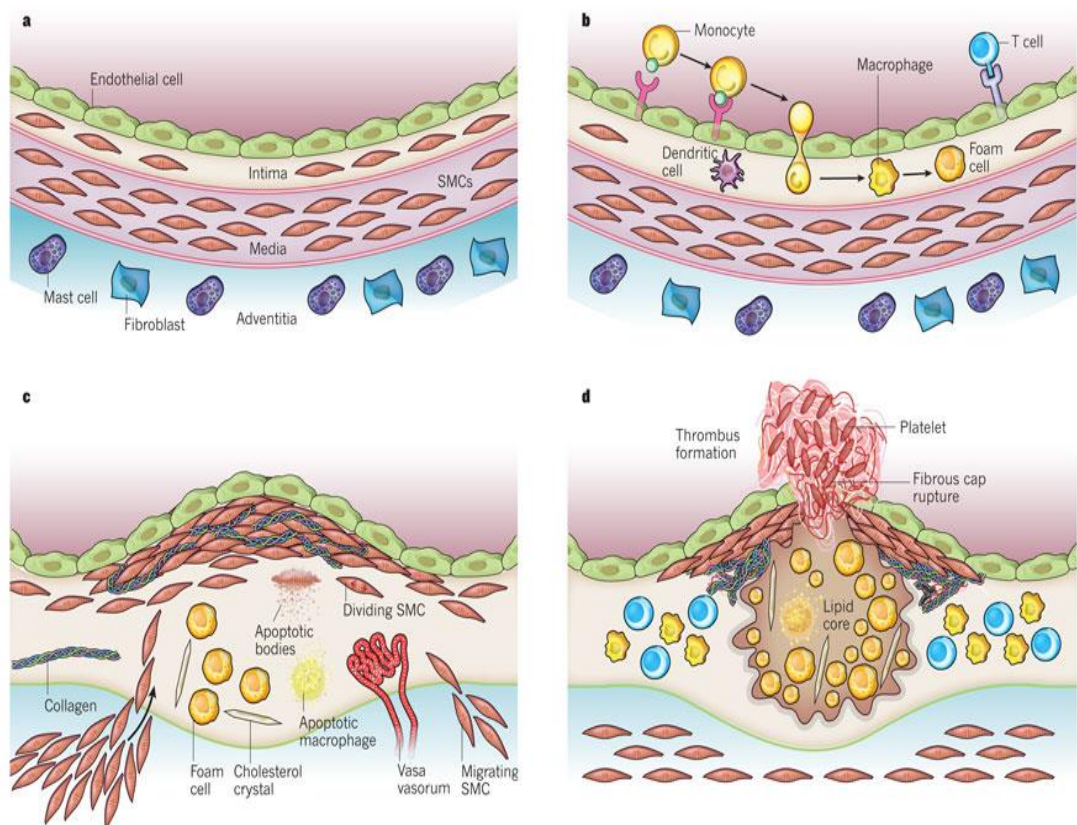


Fig.2.1. Development of atherosclerotic plaque (Libby, Ridker, & Hansson, 2011).

2.1.4. PREVALENCE OF OVERWEIGHT AND OBESITY IN THE UK

A meta- analysis of the global burden of disease reports the prevalence of overweight and obesity in the UK to be 68% for both overweight and obese men aged ≥ 20 years. The prevalence for women of same age was 59% for overweight and obese women (Ng et al., 2014). In England, most people are overweight or obese, this includes 61.3% of adults and 30% of children aged between 2 and 15 years (DH, 2013).

2.1.4.1 Adiposity and cardiometabolic disease

The pathogenesis of atherosclerosis has been attributed to a number of metabolic risk factors associated with T2DM, including obesity (Bastard et al., 2006), as obesity accounts for 80–85% of the overall risk of developing T2DM and underlies the current global spread of the condition (Frederich et al., 2015). Obesity, characterised by increased adipose are both independent risk factors of CVD and are also associated with low concentrations of serum 25(OH)D, suggesting a non-causal explanation for their association (Miettinen et al., 2014). Inflammation, a major feature of atherosclerosis is a characteristic of obesity, as adipose tissues, in addition to being a storage site for vitamin D and an energy store, secrete pro-inflammatory cytokines including IL-6, IL-8, TNF- α , and resistin that may induce a low-grade inflammatory state (Christen et al., 2016; Maury & Brichard, 2010). Adiposity is also characterised by increased circulating free fatty acids as well as decreased secretion of anti-inflammatory cytokines including adiponectin (Badawi et al., 2010; Bray, Clearfield, Fintel, & Nelinson, 2009; Van Gaal, Mertens, & De Block, 2006).

The increased secretion of fatty acids by adipocytes stimulates the production of ROS by upregulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and downregulating the expression of anti-oxidative enzymes (Goncharov, Avdonin, Nadeev, Zharkikh, & Jenkins, 2015). The increased expression of NADPH oxidase subunit p47phox in the endothelium of overweight/obese adults have been shown to promote endothelial oxidative stress compared to people with normal weight. The endothelial oxidative stress leads to systemic inflammation and endothelial dysfunction, important in the development of cardiometabolic disease (Matsuda & Shimomura, 2013; Otani, 2011; Silver et al., 2007). Due to the existing relationship between obesity and cardiometabolic disease, vitamin D deficiency may

be considered one of the mechanisms linking obesity and increased cardiometabolic disease risk. As a result of this association, it has been suggested that vitamin D deficiency be considered a non-traditional risk factor for cardiometabolic disease (Da Costa et al., 2012; James, 2008; Lu et al., 2007; Martins et al., 2014).

2.1.4.2 Associations between vitamin D and adiposity

Associations between vitamin D deficiency, adiposity and cardiometabolic health have been reported (Bray & Bellanger, 2006; Turer, Lin, & Flores, 2013), as vitamin D synthesis, action and storage modulate and are modulated by adiposity, since human adipocytes express VDR, CYP3A4, CYP27B1 and the catabolic CYP24A1 (Earthman, Beckman, Masodkar, & Sibley, 2012; Ellero et al., 2010; Romacho, Elsen, Rohrborn, & Eckel, 2014). A potential mechanism by which vitamin D may interact with adipose tissue may be by hindering lipid accumulation and important adipogenic transcription factors during adipocyte differentiation (Ding, Gao, Wilding, Trayhurn, & Bing, 2012). There is evidence that vitamin D impedes the differentiation of pre-adipocytes to mature adipocytes *in vitro* by suppressing the expression of key activators of adipogenesis such as the CCAAT/enhancer binding protein alpha (C/EBP α) and peroxisome proliferator-activated receptor gamma (PPAR γ) (Kong & Li, 2006). These activators induce the expression of various genes associated with lipogenesis, lipolysis and insulin sensitivity including glucose transporter (GLUT 4) and fatty acid synthase (Madsen, Siersbaek, Boergesen, Nielsen, & Mandrup, 2014). Thus, by inhibiting C/EBP α mRNA expression and PPAR γ activity, vitamin D also inhibits fatty acid synthase (FAS) enzyme expression resulting in reduced lipid accumulation (Madsen et al., 2014). Vitamin D has also been reported to inhibit chronic inflammation in adipose tissues (Abbas, 2016). A study in preadipocytes isolated from human subcutaneous white adipocytes has shown reductions in TNF- α , MCP-1 after vitamin D₃ treatment (Lorente-Cebrian et al., 2012). In cultured human adipocytes vitamin D₃ could be anti-inflammatory, as it decreases macrophage induced chemokines, cytokines release by adipocytes and the chemotaxis of monocytes by inhibiting the NF κ B and MAPK signalling pathways (Ding, Wilding, & Bing, 2013).

2.1.4.3 Observational studies

Several studies have shown associations between obesity and vitamin D deficiency (Blum et al., 2008; Earthman et al., 2012; Gonzalez-Molero et al., 2013; Nwudu, 2015; Pereira-Santos, Costa, Assis, Santos, & Santos, 2015; Vimalaswaran et al., 2013). A recent UK cross-sectional observational study performed in autumn and spring in 223 normal-weight, overweight, and obese adults aged 25-75 years, found that mean serum 25(OH)D was lower in overweight (45nmol/L) and obese subjects (40.8nmol/L) compared to normal weight (58.6 nmol/L) subjects. This study also found an inverse association between serum 25(OH)D with BMI ($r = -0.296$, $P < 0.001$) in autumn, spring and winter, and for every 5 unit increase in BMI, 25(OH)D decreased by 12.3% (95% CI: 7.7%, 16.6%; $P < 0.001$) (Walsh et al., 2016). A meta-analysis of 23 observational studies performed in Brazil by Pereira-Santos et al., reported vitamin D deficiency to be higher in obese subjects compared to normal weight subjects (PR: 1.35; 95% CI: 1.21–1.50) and 24% higher than in the overweight group (PR: 1.24; 95% CI: 1.14–1.34) regardless of age and threshold used to define vitamin D deficiency (Pereira-Santos et al., 2015).

2.1.4.4 RCT

In contrast to the findings from the above observational studies, a 26 week RCT performed in Denmark did not find a significant effect of 7,000 IU (175µg)/d vitamin D₃ on body fat, subcutaneous and visceral adipose tissue in 52 healthy obese adults aged 18-50 years with BMI > 30kg/m² and a plasma 25(OH)D concentration <50 nmol/L in both treatment groups (Wamberg, Kampmann, et al., 2013). The high dose vitamin D improved plasma 25(OH)D concentration from 33nmol/L to 110nmol/L, but was unable to reduce lipid accumulation, this may suggest that vitamin D treatment is unable to inhibit fatty acid synthase enzyme as reported (Madsen et al., 2014).

2.1.5 VITAMIN D AND CARDIOMETABOLIC RISK

Vitamin D deficiency or insufficiency is associated with an increased risk of cardiometabolic disease and progression of atherosclerosis (Kassi, Adamopoulos, Basdra, & Papavassiliou, 2013). The role of vitamin D in CVD pathogenesis mostly arises from observational studies, one of which reported a rise in CV events at

increased distance from the equator during winter (Motiwala & Wang, 2012). The first observation was from rats placed on a vitamin D deficient diet for 9 weeks. The rats showed raised systolic blood pressure (SBP) and enhanced ventricular and vascular muscle contractile function, suggesting a role of vitamin D deficiency in CVD (Weishaar & Simpson, 1987). There is also evidence from a community based case control study in 179 MI patients with age and sex matched controls. In this study, the MI patients had significantly lower mean 25(OH)D concentrations than controls (32.0 vs. 35.5 nmol/L, $P = 0.017$), and the case-control differences were greatest during winter and spring (Scragg, Jackson, Holdaway, Lim, & Beaglehole, 1990). These evidence have been considered to be adequate to establish vitamin D deficiency as a CVD risk factor (Kienreich, Grübler, et al., 2013; Weyland, Grant, & Howie-Esquivel, 2014).

Vitamin D exerts multiple vaso-protective effects by improving endothelial dysfunction, inhibiting VSMC proliferation and migration, downregulating the inflammatory process (Wang et al., 2013), and indirectly protects against atherosclerosis by decreasing insulin resistance, improving lipid profile and blood pressure parameters (Lee et al., 2008). Inverse associations between vitamin D and cardiometabolic disease risk (Elamin et al., 2011; Pittas et al., 2010), including inflammation (Ngo, Sverdllov, McNeil, & Horowitz, 2010), insulin resistance (Afzal, Bojesen, & Nordestgaard, 2013; Mezza et al., 2012), hypertension (Jungert, Roth, & Neuhauser-Berthold, 2012), dyslipidaemia (Wang, Xia, Yang, & Peng, 2012), endothelial dysfunction (Muscogiuri et al., 2012) oxidative stress (de Almeida et al., 2016) and arterial stiffness (Rodriguez, Scott, Srikanth, & Ebeling, 2016; Sunbul et al., 2016) have been reported. These associations arise from evidence that all the pathways and cell types involved in CVD are modulated by vitamin D metabolites, as most CV and inflammatory cells express VDR and CYP27B1 (Dickie et al., 2010; Krivosikova, Gajdos, & Sebekova, 2015). Since these cardiometabolic risk factors are the primary stimulants for the development of atherosclerosis, it is therefore important to know the effect of vitamin D on each of these cardiometabolic risk markers.

2.1.6. VITAMIN D AND ENDOTHELIAL DYSFUNCTION

The endothelium is a significant vessel wall component involved in the initiation and progression of atherosclerosis, as impaired endothelial function is the first abnormality demonstrated by the vessel wall (Endemann & Schiffrin, 2004; Kassi et al., 2013; Nadar, Blann, & Lip, 2004). Endothelial cells are single layered cells that line the inner surface of blood vessels and are the structural and functional barriers between blood and the vessel wall and they modulate both vascular tone and inflammation (Marti et al., 2012; McGorisk & Treasure, 1996; Munzel, Sinning, Post, Warnholtz, & Schulz, 2008). Endothelial cells prevent platelet and leukocyte adhesion and aggregation whilst regulating blood flow (Marti et al., 2012), and maintain vascular health by secreting nitric oxide (NO) and prostacyclin which exerts anti-aggregatory effects on platelets. Additionally, endothelial cells also secrete heparin and protein that exerts anti-coagulatory or fibrinolytic effects and as well suppressing proliferation of the vessel wall (MacLaren & Stringer, 2007).

2.1.6.1 Pathomechanisms of endothelial dysfunction

Endothelial dysfunction is the result of an imbalance between the secretion of vasodilators and vasoconstrictors by the endothelium (Muniyappa & Yavuz, 2013). Endothelial dysfunction is characterised by a change in the properties of endothelial cells towards reductions in the potent vasodilator NO, increased production of ROS and the development of pro-inflammatory and prothrombotic states (Kassi et al., 2013; Widlansky, Gokce, Keaney, & Vita, 2003). Endothelial dysfunction reduces the anti-coagulatory property of endothelial cells and increases endothelial production of plasminogen activator inhibitor-1 and tissue factor, an inflammatory response gene implicated in the increased risk of thrombosis and atherosclerosis (Brewer et al., 2011; Ha, Oh, & Lee, 2009; McGorisk & Treasure, 1996).

2.1.6.2 Proposed effects of vitamin D on the endothelium

Vitamin D is essential in the maintenance of a stable endothelium due to the presence of both CYP27B1 and VDR on endothelial cells (Dalan, Liew, Tan, Chew, & Leow, 2014). CYP27B1 and VDR both upregulate the vascular endothelial growth factor (VEGF) which increases NO synthesis (Norman & Powell, 2005) and inhibits the increased expression of p22 (phox), NADPH oxidase (the primary enzyme that

produces ROS). CYP27B1 and VDR also improve the coupling of endothelial NO synthase, thus decreasing the production of ROS (Kassi et al., 2013; Norman & Powell, 2005). Vitamin D maintains vascular tone by regulating the calcium dependent secretion of endothelium derived contracting factors, a vasoconstrictor metabolite of arachidonic acid (Wallis, Penckofer, & Sizemore, 2008). As a result of decreasing calcium influx into endothelial cells, vitamin D decreases the production of endothelium-derived contracting factor (Bischoff-Ferrari et al., 2010). The calcium independent mechanism by which vitamin D decreases the production of endothelium derived contracting factors is by downregulating cyclooxygenase-1 expression, the primary source of endothelium derived contracting factors (Melamed, Michos, Post, & Astor, 2008).

Vitamin D protects the endothelium from the damaging effects of advanced glycation end products (AGEs) by suppressing the expression of RAGE, IL-6 and IL-8 (Zitman-Gal, Golan, Green, Bernheim, & Benchetrit, 2012). In addition to suppressing the migration and proliferation of endothelial cells (Mantell, Owens, Bundred, Mawer, & Canfield, 2000), vitamin D also decreases platelet activation and the expression of vascular adhesion markers on endothelial cells (Dalan et al., 2014). Through the anti-proliferative effect of both endothelin and epidermal growth factor, vitamin D may also modulate both VSMC function and homeostasis as VSMCs express the VDR and CYP27B1 (Kassi et al., 2013). Vitamin D modulates VSMC morphology by stabilising its musculo-elastic multilayer whilst increasing elastin generation through the regulation of vessel wall scaffolding proteins including collagen type 1, elastin and matrix metalloproteinase-9 (Norman & Powell, 2005). Vitamin D affects endothelial function by indirectly reducing blood pressure by suppressing the RAS (Li et al., 2002) or by reducing vascular resistance (Norman & Powell, 2005). VDR signalling represses atherosclerosis to some extent by impeding the upregulation of the local RAS in macrophages (Li, 2013).

2.1.6.3 Observational studies

Observational studies have established an association between serum 25(OH)D concentrations and endothelial dysfunction. In 23 asymptomatic vitamin D deficient (25(OH)D <25nmol/L) adults with mean (\pm SD) age of 23.3 ± 3.0 years, endothelial function determined by flow mediated dilatation (FMD) measurements were

significantly lower in the vitamin D deficient participants compared to vitamin D sufficient controls (25(OH)D = 75nmol/L). In this study, the low serum 25(OH)D concentrations in the vitamin D deficient participants was positively associated with FMD ($r = 0.45$; $P = 0.001$) (Tarcin et al., 2009). Another study in 81 early Parkinson's disease (PD) patients with mean (\pm SD) age of 67.4 ± 8.1 and 52 age matched healthy controls, found lower serum mean (\pm SD) 25(OH)D concentrations of 54.5 ± 23.8 vs. 63.0 ± 23.3 nmol/L, ($P < 0.05$) and FMD 7.1 ± 1.8 vs. 8.1 ± 2.1 %, ($P < 0.05$) in PD patients' than in the healthy controls. Low serum 25(OH)D concentrations in the PD patients was positively associated with FMD ($R^2 = 0.331$, $\beta = 0.494$, $P < 0.001$) (Yoon, Park, Yong, & Hong, 2015).

2.1.6.4. RCTs

Several RCTs have evaluated the effect of vitamin D supplementation on endothelial function but results have been inconsistent. In a US cohort of 45 overweight and obese African Americans, mean (\pm SD) age 31 ± 2 years and baseline plasma 25(OH)D concentrations of 34.3 ± 2.2 nmol/L, 60,000IU, (1500 μ g) vitamin D₃ was administered every 4 weeks for 16 weeks resulted in significant reductions (54 ± 6 s - 43 ± 3 s, $P = 0.02$) in mean \pm (SD) FMD in the vitamin D group (Harris et al., 2011). Similarly, another 4 months UK RCT in 55 stroke patients with mean (\pm SD) age 72.9 ± 8.7 years and baseline 25(OH)D concentration of 40.8 ± 16.8 nmol/L, found significant reductions in mean (\pm SD) FMD from baseline to week 8 (6.9 ± 3.5 % vs 3.7 ± 3.1 %, $P = 0.007$) after receiving a single large dose of 100,000IU (2500 μ g) vitamin D₂ compared to placebo (Witham, Dove, Sugden, Doney, & Struthers, 2012). However, contrary to these findings, a US study in 84 non-diabetic and non-hypertensive overweight or obese participants with a baseline plasma 25(OH)D concentrations <50 nmol/L and a mean (\pm SD) age of 39 ± 13 years, did not find significant improvement with the weekly administration of 50,000IU (1250 μ g) vitamin D₂ for 8 weeks on endothelial-dependent vasodilation (EDV) determined by brachial artery ultrasound. In this study plasma 25(OH)D concentrations increased from a median (range) value of 37.5 (28.5-43.8) nmol/L at baseline to 66.3 (57.5-81.8) nmol/L at 4 weeks and 71.8 (63.5-90.8) nmol/L at 8 weeks in the vitamin D group (Borgi et al., 2016). Similarly, findings from a RCT conducted in Singapore did not show significant improvement on endothelial function assessed by reactive hyperaemia index (RHI) after administering 4,000IU(100 μ g/d) vitamin D₃ for

16 weeks to 64 multi-ethnic vitamin D deficient T2DM patients with baseline serum $25(\text{OH})\text{D} \leq 50 \text{ nmol/L}$ and mean ($\pm\text{SD}$) age of 53.5 ± 9.5 years (Dalan et al., 2016). The reason for the non-significant improvement in this study may be due to the fact that participants already had advanced T2DM.

2.1.7. VITAMIN D, INFLAMMATION AND CARDIOMETABOLIC RISK

Inflammation plays a major role in all phases of atherogenesis (Wong, Meredith, Lin, & McManus, 2012). Inflammation mechanisms mediate the gradual advancement of atherosclerosis and its various complications, as both innate and adaptive immune responses are implicated in the atherogenic process (Kassi et al., 2013; Ross, 1999). T cells including T helper (Th)1, Th2, Th17, T regulatory cells, B cells, in addition to leucocytes and transformed monocytes have been reported to be present in atherosclerotic lesions (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011). Th1 is responsible for activating the inflammatory response observed in the atherogenic process, as it stimulates the secretion of pro-atherogenic cytokines including IL-6, IL-12, interferon- γ and TNF- α (Danik & Manson, 2012; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011).

2.1.7.1 Role of vitamin D in inflammation

Macrophages, dendritic and activated T cells express both VDR and CYP27B1 enabling vitamin D to regulate the proliferation, function and differentiation of inflammatory and immune cells (Guillot, Semerano, Saidenberg-Kermanac'h, Falgarone, & Boissier, 2010; Lahoute, Herbin, Mallat, & Tedgui, 2011). Vitamin D may decrease the secretion of pro-inflammatory cytokines by stimulating Th2 to secrete anti-inflammatory cytokines including IL-5, IL-10 and IL-13 which counteract the Th1 pro-inflammatory response (Cantorna, Zhu, Froicu, & Wittke, 2004). Vitamin D has been shown to suppress the secretion of IL-6 or TNF α in monocytes through the inhibition of p38 MAP kinase by stimulating MAPK phosphatase-1 which dephosphorylates and therefore decreases its activation (Zhang et al., 2012). Vitamin D also upregulates IL-10 secretion, and a deficiency in IL-10 has been associated with acute atherosclerosis in experimental animals (Cohen-Lahav, Shany, Tobvin, Chaimovitz, & Douvdevani, 2006; Mallat et al., 1999).

2.1.7.2 Observational studies

Vitamin D deficiency has been reported to be associated with an increase in circulating inflammatory proteins (Ngo et al., 2010; Peterson & Heffernan, 2008). A deficiency in vitamin D has been associated with vascular endothelial inflammation, elevated NFkB signalling-related suppression of vascular endothelial function, and decreased vascular endothelial VDR and CYP27B1 expression (Jablonski, Chonchol, Pierce, Walker, & Seals, 2011). Inflammation has been shown to reduce with increasing 25(OH)D concentrations, as inflammation increases vitamin D catabolism (Tolosa, Cole, Gladman, Ibanez, & Urowitz, 2010).

2.1.7.3 RCTs

In recent years, RCTs which have investigated the effect of vitamin D supplementation has been increasing. A 12 month German RCT showed that a daily dose of 3,332IU (83.3µg) vitamin D₃ significantly decreased serum mean (±SD) TNF-α concentration from baseline 7.8 ± 3.2 to -7.0 ± 2.3 pg/mL, ($P = 0.049$) in 165 healthy overweight/obese subjects with mean (±SD) baseline 25(OH)D concentration of 30.0 ± 17.5 nmol/L and age of 47.4 ± 10.3 years (Zittermann et al., 2009). Additionally, a 9 week RCT conducted in Iran reported a significant decrease in serum mean (±SD) hs-CRP concentrations from 4.5 ± 0.6 at baseline to 3.1 ± 0.5 µg/ml, ($P = 0.01$) post intervention in the vitamin D group after administering 400IU (10µg)/d vitamin D₃ in 48 healthy pregnant women aged 18-40 years at 25 weeks of gestation with mean (±SD) baseline 25(OH)D concentration of 17.8 ± 1.3 µg/L (Asemi, Samimi, Tabassi, Shakeri, & Esmailzadeh, 2013). Furthermore, a UK RCT which administered a single large dose of 100,000 IU (2500 µg) vitamin D₃ at baseline, 8 and 26 weeks to 74 patients with a history of MI and mean (±SD) age of 25.3 ± 4.2 years and baseline 25(OH)D concentration of 44.5 ± 3.3 nmol/L, found vitamin D₃ significantly decreased mean (±SD) CRP by -1.3 ± 5.4 mg/L in the vitamin D group vs. $+2 \pm 6.8$ mg/L in the placebo group, ($P = 0.03$). The 74 MI patients had a (Witham et al., 2013).

In contrast, a recent Iranian RCT did not find a significant effect of 50,000IU (1250µg) vitamin D₃ for 8 weeks on serum hs-CRP in 40 patients with major depressive disorder compared with the placebo (Sepehrmanesh et al., 2016). Mean (±SD) baseline serum 25(OH)D concentration, BMI and age was 9.2 ± 6.0 µg/L,

26.9 ± 3.8 kg/m² and 36.1 ± 6.9 years respectively for the patients. This study was unable to examine whether the favourable effects of vitamin D supplementation were greater in vitamin D deficient individuals than in those without a deficiency. A systematic review of 11 RCTs which administered vitamin D orally in any dose, duration and form among adult overweight and obese participants aged ≥18 years demonstrated that there was no effect of vitamin D supplementation on inflammatory markers (Zuk, Fitzpatrick, & Rosella, 2016). Besides being limited by a small number of eligible studies, most of the included trials were aimed at investigating the effect of vitamin D supplementation on body weight, muscle function and skeletal outcomes, thus, most included studies were not originally designed to investigate the effect of vitamin D on inflammatory markers (Zuk et al., 2016). A meta-analysis of 13 RCTs which supplemented with a dose ranging between 1000-12000 IU/d and a study duration of 12-52 weeks in overweight and obese subjects showed that supplementation with vitamin D did not affect CRP, TNF-α and IL-6 in the obese and overweight subjects between the age range of 9-75 years (Jamka, Woźniewicz, Walkowiak, et al., 2015). This meta-analysis was limited by 4 RCTs that combined vitamin D and calcium supplementation, which makes it difficult to draw adequate conclusions on the effect of vitamin D in the analysed studies due to calcium effect. Additionally, study durations were different and could influence the full interpretation of the collected data (Jamka, Woźniewicz, Walkowiak, et al., 2015).

2.1.8. VITAMIN D AND INSULIN SECRETION/INSULIN RESISTANCE

The development of atherosclerosis is accelerated by T2DM (Chait & Bornfeldt, 2009). T2DM is defined by altered insulin secretion, reduced glucose tolerance, severe hyper-glycaemia and complications that arise from the stimulation of pro-oxidants (Heshmat et al., 2012). Insulin resistance (IR) is defined as the resistance of target organs to the effects of insulin on glucose metabolism and absorption. Insulin resistance is characterised by partial inhibition of hepatic glucose output and weakened insulin-mediated glucose uptake in skeletal muscle and adipose, resulting in increased insulin requirements (Kahn & Flier, 2000; Pittas, Joseph, & Greenberg, 2004). Associations have been found between vitamin D deficiency and insulin resistance, as pancreatic β cells that secrete insulin express both VDR and CYP27B1, enabling it convert 25(OH)D to 1,25(OH)₂D₃ (Bland et al., 2004; Deleskog et al., 2012; Forouhi et al., 2012; Martin & Campbell, 2011).

Since insulin secretion depends on calcium, and as vitamin D regulates calcium flux and intracellular cytosolic calcium pool within pancreatic β cells, vitamin D deficiency may decrease the capability of calcium to affect insulin secretion (Chiu, Chu, Go, & Saad, 2004; Pittas, Lau, Hu, & Dawson-Hughes, 2007). Vitamin D regulation of calcium flux is essential for insulin-mediated intracellular processes in insulin-responsive tissues particularly adipose tissues (Williams, Caterson, Cooney, Zilkens, & Turtle, 1990), as insulin is the main regulator of essentially all stages of adipocyte biology, hence, T2DM risk increases as body fat increases (Kahn & Flier, 2000). Vitamin D plays vital roles in glucose metabolism by enhancing both insulin exocytosis and absorption of glucose by peripheral tissues, directly stimulating insulin receptor and improving insulin resistance (Bajaj et al., 2014; Mitri, Dawson-Hughes, Hu, & Pittas, 2011). Gene polymorphisms of the DBP, VDR, or CYP27B1 genes may affect insulin release and result in insulin resistance which in turn disrupts vitamin D synthesis, transport and action (Blanton et al., 2011).

2.1.8.1 The inflammatory role of vitamin D in T2DM

T2DM, apart from being a vascular and hyperglycaemic disease, is an inflammatory disease (Taha et al., 2014), as increased pro-inflammatory cytokines are involved in β -cell dysfunction and elicit β -cell apoptosis (Pradhan, 2007). Vitamin D deficiency induces TNF- α pro-inflammatory and NF- κ B actions by downregulating the inhibitor of NF- κ B (I κ B) and increasing I κ B phosphorylation (Baker, Hayden, & Ghosh, 2011; Chagas, Borges, Martini, & Rogero, 2012; Cohen-Lahav et al., 2006; Riachy et al., 2002). Due to its immune-modulating properties vitamin D may directly and/or indirectly reduce the effects of systemic inflammation observed in T2DM patients in various ways, firstly, by inhibiting NF- κ B pathway, a major transcription factor for TNF- α and secondly by inhibiting the pro-inflammatory cytokines responsible for mediating acute inflammation and fibrogenesis (Chagas et al., 2012; Cohen-Lahav et al., 2007; Flores, 2005; Takiishi et al., 2010). Additionally, vitamin D represses lymphocyte multiplication, dendritic cell differentiation, foam cell formation and cholesterol absorption by macrophages (Oh et al., 2009; van Etten & Mathieu, 2005), by reducing the expression of toll like receptors (TLR) (Ahmad et al., 2012; Calton, Keane, Newsholme, & Soares, 2015). TLR is a transmembrane protein that initiates classical signalling cascades leading to the activation of transcription factors, including NF κ B and cytokine production (Ahmad et al., 2012; Calton et al., 2015)

2.1.8.2 Vitamin D and insulin sensitivity

The promoter region of the insulin receptor gene consists of VDRE suggesting a direct modulation of its transcription by CYP27B1 (Bland et al., 2004). It is therefore expected that vitamin D may improve insulin sensitivity by directly stimulating the expression of insulin receptors, which improves insulin responsiveness for glucose transport/insulin sensitivity (Maestro, Campion, Davila, & Calle, 2000). Vitamin D may also stimulate the peroxisome proliferator-activated receptor delta (PPAR- δ), a transcription factor that modulates fatty acid metabolism in adipose tissue and skeletal muscle (Dunlop et al., 2005).

2.1.8.3 Observational studies

Several cross-sectional studies have shown inverse associations between low serum 25(OH)D concentrations and insulin resistance (Chiu et al., 2004; Liu et al., 2009; Zhao, Ford, & Li, 2010). A cross-sectional study in 668 elderly subjects aged 70-74 years, with serum 25(OH)D \leq 50 nmol/L had a higher risk of developing T2DM. Serum 25(OH)D concentrations $>$ 50 nmol/L was inversely associated with several indicators of glucose metabolism, including HbA1c ($r = -0.10$; $P = 0.01$), fasting plasma insulin ($r = -0.10$; $P = 0.01$), HOMA-IR ($r = -0.10$; $P = 0.01$), and HOMA-B ($r = -0.10$; $P = 0.01$) (Dalgard, Petersen, Weihe, & Grandjean, 2011).

2.1.8.4 RCTs

Several RCTs have been performed using various doses, time scales and populations and some of these RCTs have reported that vitamin D supplementation improves insulin resistance. A 6 months RCT conducted in New Zealand in 81 insulin resistant South Asian women, aged 23-68 years with baseline serum 25(OH)D concentration $<$ 50nmol/L found that 4,000IU (100 μ g)/d vitamin D₃ caused a significantly reduced median (range) HOMA1-IR by -20.3 (0.2, 20.8) in the vitamin D group and an increased HOMA-IR +0.4 (1.2, 20.4), ($P = 0.03$) in the placebo group. There was also a significant difference in change in fasting serum median insulin between groups with a decrease of -11.2 (7.9, 11.9) in the vitamin D group and an increase of +13.1 (10.2, 17.3), ($P = 0.02$) in the placebo group (von Hurst, Stonehouse, & Coad, 2010). Similarly a 6 weeks Indian RCT which administered 120,000IU (3,000 μ g) vitamin D₃ fortnightly to 71 healthy centrally obese men aged \geq 35 years with mean

(\pm SD) baseline serum 25(OH)D concentration 36.5 ± 14.6 nmol/L, showed significant difference in changes in oral mean (\pm SD) glucose insulin sensitivity by 21.2 ± 67.8 in the vitamin D group to -11.4 ± 60.9 , ($P = 0.038$) in the placebo group (Nagpal, Pande, & Bhartia, 2009). In addition, a 9 week Iranian RCT which administered 400 IU (10 μ g) vitamin D₃ in 48 healthy pregnant women aged 18-40 years at 25 weeks of gestation and a baseline mean (\pm SD) serum 25(OH)D concentration of $17.8 \pm 1.3\mu$ g/L, showed a significant decrease in mean insulin concentrations in the vitamin D but not in the placebo groups -1.0 vs. $+2.6$ μ IU/mL, ($P = 0.04$). This study also found a significant decrease in fasting mean plasma glucose (vitamin D vs. placebo groups: -0.65 vs. -0.12 mmol/L, ($P = 0.01$) (Asemi, Samimi, et al., 2013).

Contrary to studies which demonstrated significant effects of vitamin D supplementation on glucose homeostasis parameters, some studies have found no significant effects. A 12 week US RCT which administered either 400IU (10 μ g) or 2,000 IU (50 μ g) /d of vitamin D₃ showed no significant effect of both doses on β -cell function and insulin action in 51 obese adolescents, mean (\pm SD) age 15.0 ± 1.9 years and baseline serum 25(OH)D concentration of $24.0 \pm 8.1\mu$ g/L (Javed et al., 2015). The obese adolescents recruited for this study were vitamin D sufficient with no prediabetes or diabetes and the small increase in 25(OH)D concentration of 3.1 μ g/L in the group treated with daily 2,000IU vitamin D₃ compared with the 0.8 μ g/L increase in the group treated with 400 IU/d may not be sufficient for generating marked changes in insulin sensitivity. Additionally, a 12 week RCT performed in Denmark did not show significant improvement in insulin sensitivity in 16 T2DM patients with mean (\pm SD) age of 61.6 ± 4.4 years and baseline serum 25(OH)D concentration of 31.0 ± 4.9 nmol/L, compared to the placebo group following the administration of 11,200 IU (280 μ g)/d for 2 weeks and 5,600 IU (140 μ g/d vitamin D₃ for 10 weeks (Kampmann et al., 2014). Even though supplementation increased patients' vitamin D concentrations to recommended levels, insulin sensitivity did not significantly improve, as patients in this study already had established and widespread CVD. The established CVD could have led to irreparable anatomical changes in the vascular wall, enough to prevent positive impact of the active treatment.

2.1.9. VITAMIN D AND OXIDATIVE STRESS

Atherosclerosis results from the oxidative modification of LDL-C in the arterial wall by ROS, and common risk factors for atherosclerosis increase the risk of the production of free ROS from endothelial, vascular smooth muscle and adventitial cells (Vogiatzi, Tousoulis, & Stefanadis, 2009; Vural et al., 2012). Under normal physiological conditions there is balance between the production of free oxygen radicals and the antioxidants defence mechanisms against toxicity. However, an imbalance between oxidants and antioxidants results in cellular damage and leads to a pro-oxidant process known as oxidative stress (Deng, Cheng, & Shen, 2016). Oxidative stress also results from increased production and/or decreased clearance of free radicals, which may alter the DNA, proteins and lipids leading to endothelial dysfunction and death by apoptosis or necrosis thus promoting atherosclerosis, cell death, tissue damage, and organ dysfunction (Haas, Jafri, Wehmeier, Onstead-Haas, & Mooradian, 2016; McCord & Edeas, 2005). Vitamin D acts as an antioxidant, thus maintaining redox homeostasis (Djordjevic, Zvezdanovic, & Cosic, 2008; Van Laer, Hamilton, & Messens, 2013), however, studies relating to the antioxidant properties of vitamin D are sparse (Nikooyeh & Neyestani, 2016). Vitamin D protects against oxidative stress by mediating the gene expression and protein synthesis of antioxidants such as catalase (CAT), superoxide dismutase and glutathione peroxidases in cell lines and animal models. Vitamin D does this by improving cell viability and reducing the production of ROS, and inhibiting cellular damage promoted by hydrogen peroxide (Benhusein, Mutch, Aburawi, & Williams, 2010; Tohari, Zhou, & Shu, 2016). Vitamin D also has the capacity to protect endothelial cells from oxidative stress by preventing superoxide anion generation, thus inhibiting ROS and hindering apoptosis (Wong, Delansorne, Man, & Vanhoutte, 2008).

2.1.9.1. Observational studies

There is evidence that low serum or plasma 25(OH)D concentrations results in increased oxidative stress (Codoner-Franch et al., 2012; Krivosikova et al., 2015). A cross-sectional clinical study in 66 obese Caucasian children with hepatitis C virus showed that vitamin D deficiency increased endothelial activation, inflammation and oxidative/ nitrosative stress (Codoner-Franch et al., 2012). Another study in rat models showed that vitamin D deficiency leads to mild oxidative stress in the

muscle, which may act as a trigger for increased proteolysis in the vitamin D deficient muscle (Krivosikova et al., 2015).

2.1.9.2. *In vitro* studies

Studies in human cell lines have also reported the protective effect of vitamin D against oxidative stress (Haas et al., 2016; Uberti et al., 2014). A study aimed at evaluating the ability of vitamin D to suppress hyperglycaemia-induced oxidative stress in human primary endothelial cell found vitamin D treatment to protect vascular endothelial cells from oxidative stress (Haas et al., 2016). Similarly, a study which used cultured human umbilical vein endothelial cells (HUVEC) undergoing oxidative stress demonstrated that vitamin D may prevent endothelial cell death by modulating the interplay between apoptosis and autophagy, resulting in the inhibition of superoxide anion generation and the stimulation of NO production (Uberti et al., 2014).

2.1.9.3 RCTs

There is evidence that vitamin D supplementation protects cells against oxidative stress mediated complications and strengthens antioxidant defences. One 8 week Iranian RCT in 40 patients between 18 and 65 years with major depressive disorder and baseline mean (\pm SD) serum 25(OH)D concentration of $9.2 \pm 6.0 \mu\text{g/L}$, showed a significant mean increase in the anti-oxidant glutathione in the vitamin D group compared to the placebo group by $+170$ vs. $-213 \mu\text{mol/L}$, ($P = 0.04$) after administering a single large dose of 50,000IU (1250 μg) vitamin D₃ (Sepehrmanesh et al., 2016). Similarly, in 53 non-alcoholic fatty liver disease patients with mean (\pm SD) age 40.3 ± 8.7 years and baseline median (range) serum 25(OH)D concentration of 28.8 (22.0-71.0 nmol/L), 50,000IU (1250 μg) vitamin D₃ every 2 weeks for 4 months significantly decreased mean oxidative stress measured by serum malondialdehyde (MDA) by -2.1 vs. -1.2 ng/mL , ($P = 0.03$) in the vitamin D compared to placebo group (Sharifi, Amani, Hajiani, & Cheraghian, 2014). Contrary to these findings, another Iranian RCT in 54 pregnant women with gestational diabetes with baseline mean (\pm SD) age of 31.7 ± 5.6 years and serum 25(OH)D concentration of $51 \pm 35.8 \text{ nmol/L}$, demonstrated that 50,000 IU vitamin D₃ for 6 weeks did not affect oxidative stress determined by total glutathione (Asemi, Hashemi, Karamali, Samimi, & Esmailzadeh, 2013). A limitation of this study is

that it did not investigate the effect of vitamin D supplementation on other biomarkers of oxidative stress such as catalase and superoxide dismutase which may have shown significant improvements.

2.1.10. VITAMIN D AND LIPID METABOLISM

Dyslipidaemia is the most important risk factor for atherosclerosis, as abnormalities in lipid metabolism, particularly in LDL-C lead to the development of atherosclerosis (Koba & Hirano, 2011). However, the association of vitamin D with lipids is the least investigated, as mechanisms through which vitamin D influences lipid metabolism are unknown (Kassi et al., 2013). Since vitamin D is synthesised from 7DHC, a derivative of cholesterol, it has been deduced that vitamin D deficiency may result in increased cholesterol synthesis (Jastrzebski, Kortas, Kaczor, & Antosiewicz, 2016).

2.1.10.1. Role of vitamin D in lipid metabolism

Vitamin D has been reported to affect lipids in several ways, and one such effect is its role in the improvement of cholesterol metabolism. A study which administered vitamin D in differentiating macrophages reported reduced acetylated and oxidised LDL-C absorption, thus decreasing foam cell formation by inhibiting cholesteryl ester formation whilst enhancing cholesterol clearance compared with vitamin D deficient macrophages (Riek, Oh, & Bernal-Mizrachi, 2013). Vitamin D is capable of inhibiting bile acid synthesis through the interaction of its VDR with farnesoid X receptor (FXR). As a result of this interaction, FGF15 is induced to a level required to inhibit the expression of Cyp7a1, the rate limiting enzyme in bile acid synthesis. The repression of cyp7a1 in turn inhibits bile acid synthesis which leads to reduced cholesterol concentrations (Gonzalez & Moschetta, 2014; Schmidt et al., 2010).

Vitamin D indirectly affects lipids, by inhibiting PTH secretion, as PTH has been reported to reduce lipolysis *in vitro* (Zemel, Shi, Greer, Dirienzo, & Zemel, 2000). Additionally, vitamin D may affect serum lipid concentration by increasing calcium concentrations which in turn may reduce hepatic TAG synthesis and/or secretion (Zittermann et al., 2009). Furthermore, the ability of vitamin D to improve both insulin secretion and sensitivity may indirectly influence lipid metabolism (Kamycheva, Jorde, Figenschau, & Haug, 2007). Decreasing the level of TAG and

LDL-C is also another mechanism by which vitamin D may protect against atherosclerosis, as vitamin D increases lipoprotein lipase activity in cultured adipocytes (Kassi et al., 2013).

2.1.10.2. Observational studies

There is evidence to show that high serum 25(OH)D concentrations lead to improvements in lipid profile compared to low serum 25(OH)D concentrations (Chaudhuri et al., 2013; Kelishadi, Farajzadegan, & Bahreynian, 2014). A review of 22 cross sectional studies found serum 25(OH)D concentration to be positively associated with serum HDL-C concentration (Jorde & Grimnes, 2011). Additionally, a longitudinal study where participants were followed for 14 years reported an inverse association between serum 25(OH)D and TAG concentrations (Jorde, Figenschau, Hutchinson, Emaus, & Grimnes, 2010).

2.1.10.3 RCTs

RCTs have also reported beneficial effect of vitamin D supplementation on lipid profile in humans. A daily dose of 3,320 IU (83µg) vitamin D₃ for 12 months significantly reduced TAG in the vitamin D compared to placebo group by -13.5 vs. +3.0%, ($P < 0.001$) in 165 overweight/obese subject with mean (\pm SD) age of 47.4 ± 10.3 years and baseline 25(OH)D concentration of 30 ± 17.5 nmol/L (Zittermann et al., 2009). In another study, 12 weeks of daily 4,000IU (100µg) vitamin D₃ administration in 44 HIV- infected people with mean (\pm SD) age of 47 ± 8 years and a baseline 25(OH)D concentration < 50 nmol/L, led to significant reductions in median TC -9.0 (-25.0-4.0) vs. -1.0 (-18-12), ($P = 0.009$) and non-HDL-C -7 (-21-8) vs. -2 (-11-11), ($P = 0.022$) in the vitamin D compared to placebo group respectively (Longenecker et al., 2012). In contrast to these findings, a 16 week RCT conducted in Norway did not find a significant effect of either 400 or 1,000 IU (10 or 25 µg) vitamin D₃ on lipid profile in 215 healthy South Asians aged 18-50 years and a baseline 25(OH)D concentration < 50 nmol/L (Madar et al., 2014). This study was designed primarily for muscular strength outcome, and the duration of the study may not have been sufficient to raise serum concentrations of 25(OH)D to affect the lipid profile markers. Additionally, the daily dose of 1,000IU (25µg) administered to vitamin D deficient individuals may have been inadequate to replete vitamin D stores and exert potential cardio-protective effects. The use of a higher daily dose e.g. 5,000

IU (125µg) in individuals with 25(OH)D concentrations <55 nmol/L has been recommended (Chel, Wijnhoven, Smit, Ooms, & Lips, 2008). Furthermore, the administration of 11,200 IU (280 µg) /d for 2 weeks and 5600IU (140 µg)/d vitamin D₃ for 10 weeks did not significantly affect lipid profile in T2DM patients with mean (\pm SD) age 61.6 ± 4.4 years and baseline serum 25(OH)D concentration 31.0 ± 4.9 nmol/L compared to the placebo group (Kampmann et al., 2014). It may be possible that vitamin D supplementation may in fact not elicit clinical/health effects on the T2DM patients, as T2DM is a disease state characterised with impaired endothelial progenitor cells or endothelial dysfunction (Fadini et al., 2005).

2.1.11. VITAMIN D AND ARTERIAL STIFFNESS

Arterial stiffness and atherosclerosis share some common pathophysiological mechanisms even though arterial stiffening reflects the degenerative changes of ECM in the media layer of the artery whilst atherosclerosis reflects degenerative changes in the intima layer of the artery (Palombo & Kozakova, 2016). Some of the shared pathophysiological mechanisms include impaired endothelial function, chronic inflammation and insulin resistance. As a result, both arterial stiffness and atherosclerosis could be regarded as 2 synergic processes that may increase the effect of each other in the development of vascular changes underlying CVD (Palombo & Kozakova, 2016). Arterial stiffness is defined as the reduced compliance/elasticity of an artery in response to pressure change along the arterial tree (Cecelja & Chowienzyk, 2012). Arterial stiffening develops from alterations in the cellular and structural elements of the vessel wall, due to an imbalance of its scaffolding proteins, particularly collagen and elastin which interact to maintain stability of the vessel wall (Cecelja & Chowienzyk, 2012).

Any disease state characterised by increased proinflammatory cytokine secretion and hypertension would cause an imbalance in these scaffolding proteins leading to overproduction of deformed collagen and reduced levels of normal elastin (Zieman, Melenovsky, & Kass, 2005). This results in the loss of compliance of the vascular wall, which triggers an increase in SBP and a decrease in diastolic blood (DBP), resulting in increased pulse pressure associated with an increase in aPWV with early wave reflections (Brunet et al., 2011). Consequently, these haemodynamic alterations lead to left ventricular hypertrophy and a decrease in coronary perfusion (London &

Guerin, 1999). In the presence of atherosclerosis, arterial stiffness is increased, thus arterial stiffness may be a useful marker of atherosclerosis of the aorta (Hopkins, Lehmann, & Gosling, 1994).

Arterial stiffness is modulated by endothelial cell signalling and VSMC tone, as the latter may be disrupted by paracrine mediators including endothelin known to regulate vascular stiffness (Zieman et al., 2005). Angiotensin II, another paracrine mediator may also alter vascular tone by stimulating collagen generation whilst reducing elastin synthesis thereby increasing oxidative stress and stimulating cytokines and vascular hypertrophy (Zieman et al., 2005). Advanced glycation end products (AGEs) increase arterial stiffness contributing to endothelial dysfunction by reducing NO production, activating both stress signals and inflammatory responses, whilst increasing the expression of vascular adhesion molecules, growth factors, free radicals, and NF- κ B (Kuzuya et al., 2001; Rojas et al., 2000).

AGEs have been reported to produce irreversible non enzymatic cross-links between collagen and elastin, making it stiffer leading to deformity (Konova, Baydanoff, Atanasova, & Velkova, 2004). Disease, aging and other exposures in most people may reduce the elastic whilst stimulating the inelastic (collagen) component of the vessel wall (Lakatta & Levy, 2003; Lantelme, Mestre, Lievre, Gressard, & Milon, 2002). Arterial stiffness is measured by PWV, a gold standard method and reliable indicator of damaged vessel wall (Colaci et al., 2012). PWV is defined as the speed at which pulse pressure or wave travels along the arterial tree, however, limitations of the method include a lack of an established reference value and the absence of a standardisation of method (Farro et al., 2012; Laurent et al., 2006).

2.1.11.1. Observational studies

Observational studies have reported an inverse association between vitamin D deficiency and indices of arterial stiffness such as PWV and AIX. One Italian observational study in 150 postmenopausal women showed reported inverse associations between 25(OH)D concentrations < 75nmol/L with aPWV ($\rho = -0.23$, $P = 0.006$) (Pirro et al., 2012). A study in 554 men and women showed that lower serum 25(OH)D concentrations was associated with increased arterial stiffness assessed by PWV ($r = -0.190$, $P < 0.001$) and AIX ($r = -0.170$, $P < 0.001$) (Al Mheid et al., 2011).

2.1.11.2 RCTs

Several RCTs aimed at assessing the effect of vitamin D supplementation on arterial stiffness have been conducted, some of which have found vitamin D supplementation to improve arterial stiffness parameters. A 6 month US RCT in 534 prehypertension patients with mean (\pm SD) age 40.4 ± 7.5 years and baseline serum 25(OH)D <62.5 nmol/L, found that 4,000 IU (100 μ g) significantly decreased mean (\pm SD) AIx and augmentation pressure by $12.3 \pm 5.3\%$, ($P = 0.047$) and 4.0 ± 1.5 mmHg, ($P = 0.02$) respectively (Zaleski et al., 2015). Additionally, the 2 week administration of 2000 IU (50 μ g)/d vitamin D₃ in 15 healthy adults aged 19-53 years, showed significant reductions in mean (\pm SD) PWV from 7.45 ± 1.55 to 6.11 ± 1.89 m/s, ($P = 0.04$) (Al-Dujaili, Munir, & Iniesta, 2016). Contrary to these findings, a meta-analysis of 7 RCTs with doses of vitamin D₃ ranging from 1,000 IU (25 μ g/d) to 120,000 IU (3,000 μ g)/m over a duration of 2-12 months showed no improvement in arterial stiffness markers such as PWV and AIx following vitamin D supplementation (Upala, Sanguankeo, Congrete, & Jaruvongvanich, 2016). The absence of a significant effect on arterial stiffness in this study may be explained by the methodological differences among the devices used to measure PWV. Consequently, this may limit the interpretation of results as previous studies have shown different outcomes using these devices (Davies, Bailey, Griffin, & Scott, 2012; Shahin, Barakat, Barnes, & Chetter, 2013). This study also used heterogeneous doses of vitamin D supplements, thus, caution should be taken into account when interpreting the data. A second meta-analysis of 18 RCTs with vitamin D₃ doses ranging from 1,000 IU to 5,700 IU/d (25-142.5 μ g) and follow up times of 1-12 months also demonstrated that vitamin D replacement had no positive effect on PWV and AIx compared with the placebo group (Rodriguez et al., 2016). The lack of access to primary data was a limitation in the statistical analyses performed in this meta-analysis, as well as the data being extracted by a single reviewer. A more recent RCT performed in Denmark which was not included in any of these meta-analyses, also showed a lack of improvement in PWV and AIX after 3,000 IU (75 μ g)/d vitamin D₃ supplementation for 16 weeks in 40 healthy normotensive adults aged > 18 years with baseline serum 25(OH)D concentration ≤ 50 nmol/L (Bressendorff et al., 2016). This study may not have found a significant effect of supplementation because of

small sample size, even though trial was large enough to detect a difference in PWV and AIx based on sample size calculations.

2.1.12. VITAMIN D AND RENIN-ANGIOTENSIN SYSTEM

The presence of hypertension and other atherosclerotic risk factors are associated with increased vascular angiotensin II generation and activity (Dzau, 2001), as angiotensin II has been implicated in the pathogenesis of atherosclerosis (Werner, Pöss, & Böhm, 2010). The RAS is a hormone system that regulates blood volume and arterial pressure and maintains the homeostatic balance between water and electrolytes. Vitamin D suppresses RAS endogenously by blocking the activity of the cyclic adenosine monophosphate (cAMP) response element in the renin gene promoter region (Li, 2003; Li et al., 2002). Vitamin D also inhibits the expression of angiotensinogen gene by blocking the NF- κ B pathway (Deb et al., 2009). The association between vitamin D and the RAS may be attributed to the fact that both angiotensin II receptors and VDR are present in the same tissues (Ferder, Inserra, Manucha, & Ferder, 2013).

2.1.12.1 Role of vitamin D in RAS

The most substantial mechanistic studies associating vitamin D with RAS activity and blood pressure regulation were performed in animal models. It has been demonstrated that VDR-knockout mice had significant increase in renin activity and circulating plasma angiotensin II concentrations (Li et al., 2002). The VDR-knockout mice used in this study developed hypertension and cardiac hypertrophy (Li et al., 2002). Another study in CYP27B1 deficient mice, also showed increased RAS activity, hypertension and cardiac hypertrophy that was attenuated with the administration of vitamin D (Xiang et al., 2005). These studies suggest that disruptions of vitamin D signalling may lead to upregulation of the RAS, hypertension and cardiac hypertrophy. In humans vitamin D deficiency has been shown to be associated with increased risk of arterial hypertension (Pilz, Tomaschitz, Ritz, & Pieber, 2009), as vitamin D deficiency upregulates the RAS, thereby contributing to cardiac hypertrophy (Forman, Williams, & Fisher, 2010).

2.1.12.2 Observational studies

Observational studies in humans have shown that lower serum 25(OH)D concentrations are associated with upregulated circulating RAS. In 50 Indian obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) hypertensive patients with mean ($\pm\text{SD}$) age of 49.5 ± 7.8 years, plasma renin activity (PRA) was higher in the vitamin D deficient ($25(\text{OH})\text{D} < 50\text{nmol/L}$) compared to the vitamin D insufficient group ($25(\text{OH})\text{D} < 50\text{-}72.5\text{nmol/L}$) (3.7 ± 0.9 vs. $3.3 \pm 0.6 \text{ ng/ml/hr}$) (Kota et al., 2011). In this study, PRA was higher in the overweight and obese compared to the lean patients (2.9 ± 0.7 and 3.6 ± 0.9 vs. $3.3 \pm 0.6 \text{ ng/ml/hr}$) (Kota et al., 2011). A cross-sectional study of 3316 patients with median (range) age of 63.5 (56.3-70.6) years referred for coronary angiography also found that baseline median (range) serum 25(OH)D concentration of 15.6 (10.1-23.0) $\mu\text{g/L}$ was inversely associated with increased plasma renin concentration ($r = -0.105$, $P = 0.013$) and increased plasma angiotensin II concentration ($r = -0.046$, $P = 0.020$) (Tomaschitz et al., 2010).

2.1.12.3 Intervention studies

Few intervention studies performed in humans have found a significant effect of vitamin D therapy on the RAS. Oral weekly 25,000IU (625 μg) vitamin D₃ for 2 months in 15 drug-free, essential hypertension patients with mean (range) age of 43.6 (22-71) years and baseline mean ($\pm\text{SD}$) serum 25(OH)D concentration of $45.8 \pm 7.0 \text{ nmol/L}$, who were consuming a constant-salt diet found a significant reduction in plasma mean ($\pm\text{SD}$) renin concentration by $13.6 \pm 3.4 \text{ pg/mL}$ vs $24.0 \pm 5.9 \text{ pg/mL}$, ($P < 0.05$) and a non-significant decrease in plasma mean ($\pm\text{SD}$) angiotensin II concentration by $9.4 \pm 1.5 \text{ pg/mL}$ vs $13.0 \pm 2.5 \text{ pg/mL}$ (Carrara et al., 2014). In contrast, a Boston intervention study that evaluated the impact of high-dose 15,000IU/d vitamin D₃ therapy on the tissue sensitivity to Ang II in obese drug free hypertensives consuming a constant-salt diet for 1 month with a mean ($\pm\text{SD}$) age of 50.0 ± 2.5 years and baseline serum 25(OH)D concentration of $45.5 \pm 4.5 \text{ nmol/L}$, did not observe any effect of therapy on circulating RAS components (Vaidya, Sun, Larson, Forman, & Williams, 2012). The absence of an effect of vitamin D therapy could be due to the short one month duration of vitamin D₃ treatment.

2.1.13. VITAMIN D AND BLOOD PRESSURE

Hypertension (>140/90 mmHg) is a significant cause of atherosclerosis and thus, a risk factor for cardiometabolic disease. A major mechanism through which vitamin D may impact BP is the RAS (Li, 2003), as the untimely stimulation of the RAS has been associated with hypertension. Vitamin D also affects blood pressure via paracrine and endocrine mechanisms in the VSMC, endothelium and cardiomyocytes (Li et al., 2002). Vitamin D treatment has been shown to reduce plasma renin activity, angiotensin II concentrations and blood pressure by decreasing the expression of renin gene through a VDR-dependent mechanism, thus decreasing both renin and angiotensin II (Zhou et al., 2008). The molecular effects of VDR activation which includes suppression of the RAS, nephroprotective actions, or improvements in endothelial/vascular function, suggest antihypertensive properties of vitamin D (Liu, Woo, Wu, & Ho, 2013). Vitamin D may also regulate BP through effects on calcium homeostasis (Bian, Ishibashi, & Bukoski, 1996) regulation of VSMC (Somjen et al., 2005) and endothelial cell (Zehnder et al., 2002) function, inflammation and insulin sensitivity (Wang et al., 2013).

2.1.13.1. Observational studies

Evidence from observational studies and meta-analyses has shown that vitamin D deficiency/insufficiency are a significant risk marker for arterial hypertension (Kienreich, Grübler, et al., 2013; Kunutsor, Apekey, & Steur, 2013). It is hypothesised that if low concentrations of vitamin D cause hypertension, then vitamin D repletion may lower blood pressure due to its antihypertensive effects. A meta-analysis of 18 observational studies of adults aged 18-96 years reported that every 40nmol/L reduction in 25(OH)D concentration was associated with a 16% increased risk of hypertension (Burgaz, Orsini, Larsson, & Wolk, 2011).

2.1.13.2 RCTs

Several RCTs investigating the effect of vitamin D supplementation on BP have been conducted. A recent UK RCT in 15 healthy participants aged 19-53 years found that administering 2000 IU (50 µg)/ d vitamin D₃ for 2 weeks significantly reduced mean (\pm SD) SBP and DBP from 115.8 ± 17.1 mmHg and 75.4 ± 10.3 mmHg at baseline to 106.3 ± 10.9 , ($P = 0.022$) and 68.5 ± 10.1 mmHg, ($P = 0.012$) respectively post

intervention (Al-Dujaili et al., 2016). Similarly, a UK RCT which compared the effect of single large doses of 100,000 and 200,000 IU (2,500 or 5,000 μ g) of vitamin D₃ in 58 T2DM patients with mean (\pm SD) age of 66.7 ± 9.7 years and baseline 25(OH)D concentration < 100 nmol/L for 16 weeks, reported a significant decrease in mean SBP in both treatment arms than in the placebo group at 8 weeks for the individuals given 100,000IU, 141.4 mmHg vs.146.4 mmHg, ($P = 0.04$) and 200,000 IU, 136.8 mmHg vs.146.4 mmHg, ($P = 0.03$) (Witham et al., 2010). A US 4-arm RCT in 283 African Americans with median (range) 51 (44-59) years and baseline 25(OH)D concentration 39.3 (26.8-58.5) nmol/L, which compared the effects of daily 1,000, 2,000, or 4,000IU (25, 50 or 100 μ g) of vitamin D₃ found a difference of -0.66 mmHg, -3.4 mmHg and -4.0 mmHg for 1,000, 2,000 and 4,000IU (-1.4 mmHg for each additional 1,000 IU $P = 0.04$) in SBP between baseline and 3 months (Forman et al., 2013).

Conflicting results on the effect of vitamin D supplementation on blood pressure have also been reported. A 12 month RCT performed in China did not find a significant effect of the daily administration of 700 IU (17.5 μ g) vitamin D in 123 vitamin D deficient metabolic syndrome patients (Yin et al., 2016). A possible reason for lack of an effect following supplementation may be attributed to low dose vitamin D administered to vitamin D deficient group as dose may have been too low to effect improvements. A UK meta-analysis of 16 RCTs with a study duration of 5-56 weeks using a vitamin D dose above 600IU (15 μ g), reported non-significant reductions in both SBP and DBP following vitamin D supplementation (Kunutsor, Burgess, Munroe, & Khan, 2014). The majority of trials included in this meta-analysis had low risk of bias, thus, findings should be interpreted with some caution, owing to the potential differences in design and population characteristics of each trial. There was substantial heterogeneity among trials of SBP, as a result it was argued whether pooled estimates should be presented rather than reporting estimates in relevant subgroups, as the presence of heterogeneity makes pooling of risk estimates data controversial. Similarly, a RCT conducted in Austria in 188 hypertensive patients with mean (\pm SD) age of 60.1 ± 11.3 years and baseline serum 25(OH)D concentrations of 55.0 ± 13.8 nmol/L, showed no significant effect of 2,800IU (70 μ g)/d vitamin D₃ on blood pressure measurements after 8 weeks of supplementation (Pilz et al., 2015). The limitations of this study include the low

prevalence of patients with severe vitamin D deficiency and the relatively short treatment period of study, as significant effects of vitamin D in populations with low vitamin D concentrations and with longer treatment or different vitamin D cannot be excluded.

2.1.14. STUDY RATIONALE

Systematic reviews of observational studies have reported that low serum 25(OH)D concentrations are inversely associated with a broad range of cardiometabolic risk factors including hypertension, dyslipidaemia, abnormal glucose homeostasis and atherosclerosis (Grandi et al., 2010; Parker et al., 2010; Pilz et al., 2011; Pittas et al., 2010; Wang, Song, et al., 2012). The few RCTs of vitamin D supplementation completed to date have failed to show a clear beneficial effect on cardiovascular events, raising questions about the causal role of vitamin D in CVD (Kienreich, Tomaschitz, et al., 2013; Theodoratou, Tzoulaki, Zgaga, & Ioannidis, 2014). As much of the observational data collected to date cannot provide strong evidence of causality, as they only draw inferences, particularly since increasing BMI leads to an inflammatory state resulting in low serum 25(OH)D concentrations and increased CVD risk. Prospective RCTs are therefore required to test the hypothesis whether high dose vitamin D₃ supplementation significantly improve vitamin D status and cardiometabolic risk markers. Therefore, a high quality double blind, randomised placebo controlled trial of a high daily dose of 5,000IU (125µg) vitamin D₃ supplementation to test whether this intervention could improve markers of vascular function, blood pressure, lipid profile, glucose homeostasis, inflammation, oxidative stress and the RAS in overweight and obese UK males was conducted.

2.1.15. RESEARCH PURPOSE

To investigate the effect of daily dose of 5,000IU (125µg) vitamin D₃ for 8 week on cardiometabolic risk markers in a cohort of overweight and obese adult males.

2.1.16. OBJECTIVES

Primary

- To determine whether a daily dose of 5,000IU (125µg) vitamin D₃ for 8 weeks would significantly improve 25(OH)D concentration in a cohort of overweight and obese adult males.
- To determine whether a daily dose of 5,000IU (125µg) vitamin D₃ for 8 weeks would significantly improve the cardiometabolic risk markers measured in a cohort of overweight and obese adult males.

Secondary

- To investigate the association between change in 25(OH)D concentration with corresponding change in the cardiometabolic risk markers measured in in a cohort of overweight and obese adult males.

2.1.17. Hypotheses

H₁: There will be a significant increase in plasma 25(OH)D following 8 weeks daily supplementation of oral vitamin D₃ at a dose of 5,000 IU (125µg/day).

H₀: There will not be a significant increase in plasma 25(OH)D following 8 weeks daily supplementation of oral vitamin D₃ at a dose of 5,000 IU (125µg/day).

H₁: Administering oral vitamin D₃ at a dose of 5,000IU (125µg/day) in overweight and obese participants for 8 weeks will significantly improve the cardiometabolic parameters measured.

H₀: Administering oral vitamin D₃ at a dose of 5,000IU (125µg/day) in overweight and obese participants for 8 weeks will not significantly improve the cardiometabolic parameters measured.

H₁: There will be an association between plasma 25(OH)D concentration and cardiometabolic risk markers.

H₀: There will not be an association between plasma 25(OH)D concentration and cardiometabolic risk markers.

2.2 METHODS

2.2.1 Study design for vitamin D supplementation study

A parallel, randomised, double-blind, placebo-controlled trial was conducted in accordance with ethical guidelines for research involving human subjects according to the Helsinki Declaration (Dale & Salo, 1996). Ethical approval for the present study was obtained from the University of Chester Faculty Research Ethics Committee (REF: 855/13/AT/CSN) (appendix 1). The trial was registered at clinicaltrials.gov (NCT02359214), and conducted following the Consolidated Standards of Reporting Trials (CONSORT) statement (appendix 2) (Schulz, Altman, Moher, & Group, 2010). To limit the exposure of UVB radiation from the sun, the study was conducted between November 2014 to May 2015, and from October 2015 to January 2016. It is noteworthy that 4 participants finished in early May 2015, however, their final vitamin D concentration was not significantly different from the remaining group.

2.2.2 Sample size calculation

As most RCTs designed to investigate the effect of vitamin D supplementation on CVD markers were powered using musculoskeletal outcomes, it is therefore important that the design of the present study is based on a CVD outcome. The study by (Witham et al., 2012) was the most relevant, as it demonstrated significant improvement in FMD at week 8 of vitamin D supplementation. Mean (\pm SD) FMD was significantly higher in the vitamin D group compared to the placebo group, 6.5 ± 3.5 vs. 3.7 ± 3.1 , ($P = 0.007$) in patients with a history of stroke and with baseline serum 25(OH)D concentration below 75nmol/L. Based on this study findings, a sample size of 58 (29 per group) that allows for the detection of a treatment difference at 95% power, 0.97 effect size and 5% significance was required for the present study. To allow for 15% dropout, 33 participants were required in each treatment arm, on this basis it was required to screen at least 90 overweight and obese adult males. Sample size was calculated using G power software version 3.1 (G. Power, Düsseldorf, Germany).

2.2.3 Randomisation

Randomisation of participants to the vitamin D or placebo group was performed by the third party, with the use of a computer generated random number sequence (www.randomisation.com). The tablets were packaged into white plastic coded, tamper-proof containers, and sealed in sequentially numbered study packs by the third party. Both investigator and participants were blinded from intervention.

2.2.4 Supplement

Vitamin D₃ supplements containing 5,000IU (125µg) cholecalciferol, calcium phosphate, microcrystalline cellulose, magnesium stearate and silica was purchased from Bulk Powders, Colchester, UK. Placebo containing lactose was purchased from Placebo-world, Powys, UK. The vitamin D₃ supplements and placebo were both in white tablet form. The dose (5000 IU) of vitamin D₃ administered, has been safely used in previous intervention studies and it has been shown to significantly increase serum/plasma 25(OH)D concentration (Diamond, Wong, & Golombick, 2013; Yap et al., 2014; Yiu et al., 2013). Study participants were requested to store the study supplements at room temperature. Participants were requested to take one tablet at any time of day that was convenient, but to be consistent with the time over the 8 weeks (56 days). Participants were sent email reminders about taking supplements.

2.2.5 Assessment of compliance

The percentage of compliance of the vitamin D₃ supplement and placebo of the 49 participants who completed the intervention was assessed by counting the number of tablets left in the containers at the end of the study period. The overall compliance was high, compliance was 90% in the vitamin D group and 87% in the placebo group and participants attributed missing 2 or more tablets to forgetfulness. No adverse events were reported from taking the supplements at the end of the study.

Percentage compliance was calculated using the equation:

$$\% \text{ compliance} = (\text{actual/expected}) \times 100$$

2.2.6 Research centre

The trial took place in the Clinical Sciences laboratory within the Department of Clinical Sciences and Nutrition at the University of Chester. Prior to commencement of the trial, the primary investigator was trained in phlebotomy and basic first aid.

2.2.7 Participant recruitment

Participants were recruited from the City of Chester, United Kingdom through the following methods:

- Study posters/leaflets (appendix 3) were displayed on information boards at the library, café and lecture halls at the University of Chester.
- Advert shared within University intranet and both University and local newspaper articles.
- Through an announcement, on a local radio station.

2.2.8 Inclusion criteria

Participants were eligible if they were:

- Males, aged between 18-65 years
- Males who had a screening plasma 25(OH)D concentration of $\leq 75\text{nmol/L}$.
- Body mass index (BMI) $> 24.9\text{ kg/m}^2$
- Not taking vitamin D supplements.
- Free of clinically diagnosed cardiometabolic, renal and hepatic diseases.

2.2.9 Exclusion criteria

The following participants were excluded:

- Participants with clinical diagnosis of any cardiometabolic, renal, liver or gastrointestinal disease.
- Participants with BMI $< 24.9\text{ kg/m}^2$.

2.2.10 Clinics

Clinics were carried out in the Clinical Laboratory at the University of Chester, participants attended 4 clinics in total (each lasting 30 minutes).

- **Screening clinic:** Each participant was given a participant information sheet (PIS) (appendix 4) to read, followed by a written informed consent document (appendix 5) to sign. Participants were also requested to complete a screening questionnaire (appendix 6) after which weight and height were measured using a digital scale and wall mounted stadiometer to obtain BMI. Following this, venous blood (1mL) was collected for vitamin D status screening to confirm eligibility for the study. Eligible participants with plasma 25(OH)D concentrations < 75 nmol/L were invited to partake in the study.
- **Clinic 1 (week 0):** Weight, height, waist circumference (WC) and arterial function parameters including pulse wave velocity (PWV), augmentation index (AIx), aortic central systolic blood pressure (SBPao), return time (RT), systolic and diastolic blood pressure (SBP and DBP), heart rate (HR), mean arterial pressure (MAP), brachial pulse pressure (PP), aortic pulse pressure (PPao), and left ventricle ejection duration (ED) measurements were performed. After this, venous blood (20 mL) was collected to assess the cardiometabolic risk biomarkers. Randomised supplements in coded containers were then distributed to participants. Participants were also requested to complete a 3day food diary (appendix 7) to return at the next clinic visit.
- **Clinic 2 (week 4):** The procedure performed at clinic 1 was repeated in this clinic except for height measurement. Completed food diary was collected, and a new food diary was given to be returned at the final clinic.
- **Clinic 3 (week 8):** Repeat of clinic 2.

2.2.11 Measurements

All measurements were performed between 8am and 10am (due to participants fasting for 8 hours beforehand) at day 0 (baseline) day 28 (interim) and day 56 (post intervention).

2.2.11.1 Anthropometric measurements

Height measurement was performed using a digital wall mounted stadiometer (Seca, Hamburg, Germany) calibrated to the nearest 0.1cm precision. Participants removed shoes, stood straight facing forward, with the back of their head, shoulder blades, buttocks and heels touching the stadiometer. With feet flat and heels brought together, the head of the stadiometer was lowered so that hair was pressed flat and height was measured.

Body weight was measured using a digital scale (Seca, Hamburg, Germany) calibrated to 0.1 kg. Prior to measurement, participants were requested to remove shoes, heavy outer garments and empty pockets. Measurement was taken with participants standing still at the centre of the platform, with a gap between heels and weight equally distributed on both legs, after which weight was recorded. BMI was calculated as weight in kilograms divided by the square of height in metre (kg/m^2). Whilst participant was standing, WC was measured horizontally around the waist at the level of the umbilical cord using a tape measure.

2.2.11.2 Arterial stiffness and haemodynamic measurements

Arterial function parameters including pulse wave velocity (PWV), augmentation indices (brachial and aortic, AIX), central systolic blood pressure (SBPao), return time (RT), systolic and diastolic blood pressure (SBP and DBP), heart rate (HR), mean arterial pressure (MAP), brachial pulse pressure (PP), central aortic pulse pressure (PPao), and left ventricle ejection duration (ED) were measured non-invasively at baseline, 4 weeks (interim), and 8 weeks (post intervention) by the investigator with the use of a validated (Horvath et al., 2010) automatic oscillometric occlusive device known as the Arteriograph (TensioMed, Budapest, Hungary) Arteriograph -5-01,v 1.9.

Arterial stiffness measurement was performed in a quiet room at $22 \pm 1^\circ\text{C}$. Prior to measurement participants were given a glass of water and advised not to speak, sleep or move until measurement was complete. The distance between jugular (sternal) notch and symphysis pubis (distance between the aorta and its bifurcation) was measured with participant standing upright using a tape measure. Participants were allowed to rest for 10 minutes in a supine position, after which an appropriate cuff was placed tightly round the right arm. Three measurements were performed, each

lasting 2-3 minutes, with the mean of the last 2 readings recorded. After a measurement was taken, the standard deviation (SD) of the PWVao was consulted to inform the investigator about the quality of the measurement. If the SD PWVao was greater than or equal to 0 and less than or equal to 1.0m/s, the measurement was accepted as it was of good quality. However, if the SD PWVao was greater than 1.0m/s, or appears in red, the measurement was discarded, as it was of unacceptable quality and measurement would be repeated.

Upon operation, the simple upper arm cuff that picks up pressure signal was inflated to a pressure 35 mmHg over the SBP. This completely occludes the brachial artery in a stop flow condition, and pressure signals are transmitted through the cuff to the pressure sensor and are reported to show multiple peaks. As central pressure changes, early (direct) systolic wave (P1), late (reflected) systolic wave (P2) and diastolic wave (s) (P3) reach the upper arm and cause very small volume pressure changes in the cuff. These very small changes are recorded by a high-fidelity pressure sensor in the device and transmitted to the edge-position sensor by conduit arteries including the subclavian, axillary, or brachial. Arteriograph first measures the actual SBP and DBP, then cuff is deflated, and after few seconds cuff is inflated again, first to the actual DBP, then to the suprasystolic (actually measured systolic + 35 mmHg) pressure. Pressure signals are then recorded for 8 s -10 s, after which they are transmitted wireless to notebook PC. The data analysis is performed by the software of the device. To determine PWVao, arteriograph measures the time interval between the peaks of the direct (first) and reflected (late) systolic wave (return time – RT). The PWVao was calculated using the formula: $PWVao \text{ (m/s)} = \text{Jug-Sy (m)} / (RT/2 \text{ (s)})$. Augmentation indices were calculated for the aorta and brachial arteries using the formula: $AIx \text{ (\%)} = (P2-P1)/PP \times 100$. Where P1 is the amplitude of the first (direct) wave, P2 is the amplitude of the late (reflected) systolic wave and PP is the pulse pressure.

2.2.12 Dietary assessment

To obtain dietary information, 2 copies of a 3-day food diary was given to each participant at clinic 1 and 2 to record their eating and drinking on 2 weekdays and 1 weekend day. This includes food and drink consumed between meals or at night, they were also requested to write the day, time, description of meal or drink, portion

size and preparation method as well as brand product or recipe as appropriate. Participants were also requested to record food that came with any added vitamin or minerals. For homemade dishes, they were requested to record the name of the recipe, ingredients with the amount and cooking method at the back page of the diary. Dietary nutrient analysis software Nutritics v4.25 (Nutritics, Dublin, Ireland) was used to estimate mean daily energy, carbohydrate, protein, fat and vitamin D intake based on reported consumption from the 3-day food diary.

2.2.13 Blood sampling

A fasted (overnight of at least 8 hours) morning (between the hours of 8am and 10am) whole blood sample was collected by venepuncture by the investigator from the median cubital vein according to standard protocols (appendix 7). Blood samples were drawn using a 21g vacutainer needle into 10 mL sterile lithium heparin and EDTA tubes to avoid clotting. The collected blood samples were immediately transferred into an ice box. Plasma was separated by centrifuging for 10 min at 4°C at 2054g. The centrifuged plasma was aliquoted into tubes labelled with participants' ID number and dates, and stored at -80°C until batch analysis. The first batch analysis was performed from May to September 2015 and the final batch analysis between December and February 2016.

2.2.14 Biochemical measurements

Material Safety Data sheets and COSHH information were adhered to for each analyte. Prior to the experiments all reagents, microplate wells, and samples were brought to room temperature according to appropriate kit instruction booklet. To avoid cross contamination, pipette tips were changed during each standard preparation, and screw caps were also not interchanged. Multiple freeze thawed samples were not used, and all ELISAs were performed in duplicate. To ensure accurate results, only calibrated pipettes were used, kits were used before expiration date and reagents were not mixed with those from other sources or lots. Plasma samples which had concentrations that exceeded the highest standard were further diluted and repeated.

Prior to use of each ELISA kit, a QC was run 8 times to obtain the intra-assay CV, and the inter-assay CV was obtained from running commercial control assay. The CVs were calculated using the formula:

$$CV = \frac{S.D}{Mean} \times 100$$

2.2.14.1 Endothelial function

2.2.14.1.1 Plasma sE-selectin concentration determination

Plasma sE-selectin concentration (collected in heparin tubes) was determined using a quantitative sandwich enzyme immunoassay quantikine solid-phase ELISA kit (R&D Systems Europe, Abingdon, UK). The 96 well plates were pre-coated with a monoclonal antibody specific for naturally occurring human sE-selectin. Prior to assay, all samples were diluted 10-fold (30 µL of sample and 270 µL of calibrator diluent) to yield a final volume of 300 µL. Wash buffer (20 mL) concentrate was diluted by adding 480 mL of deionised water to yield a final volume of 500 mL. Substrate solutions (Colour A+B) were mixed together in equal volumes within 15 minutes of use and protected from light. The standard was reconstituted with 1.0 mL of deionised water and allowed to sit for at least 15 minutes before making serial dilutions.

Following sample and reagent preparation, assay diluent (100 µL) was pipetted into each well. Standard, control, and sample (100µL each) were pipetted into appropriate wells, which were covered with adhesive strip and incubated for 2 hours. Following incubation, wells were decanted and washed four times manually with 400 mL wash buffer. After the final wash, the microplate was inverted and blotted dry against absorbent paper towels. Conjugate (200 µL) was pipetted into each well and incubated for another 2 hours. After incubation, wells were washed four times and blotted dry against absorbent paper towels. Substrate solutions (colour A+B) (7 mL each) were mixed thoroughly, after which, 200 µL each of the mix was pipetted into wells, the microplate was covered with foil paper to protect from light and left to incubate for 30 minutes. Following incubation, 50 µL of stop solution was pipetted into each well and plate was tapped gently on bench top to ensure thorough mixing. The optical density of each well was determined within 30 minutes at 450nm

(primary) and 570nm (reference) and the sE-selectin concentration was obtained from the standard curve (see appendix 8 for example standard curve). Obtained concentrations were multiplied by the corresponding dilution factor. Intra and inter assay coefficient of variation (CV) were 3.4% and 3.4% respectively.

2.2.14.2 Oxidative stress

2.2.14.2.1 Plasma 8 - Isoprostane concentration determination

A competitive *in vitro* immuno-enzymatic assay was used for the determination of 8-isoprostane concentration (Abcam, Cambridge, UK) in plasma. Prior to the assay, 10 x wash buffer was mixed with a stir bar whilst applying low gentle heat until a clear colorless solution was obtained. Following this, 25 mL wash buffer solution was diluted with 225 mL of deionised water to yield a final volume of 250 mL. One vial of horseradish peroxidase (HRP) conjugate (12 µL) was diluted with 12 mL of HRP buffer to prepare the 1x HRP conjugate. Sample dilution (25 mL) buffer was diluted with 225 mL deionised water to yield a final volume of 250 mL. The enclosed 2 µL standard vial filled with inert gas was first centrifuged, before 1.998 mL of 1 x sample dilution buffer was added to obtain a 2 mL stock solution. Several serial dilutions were made from the stock solution

Following reagent preparation, 200 µL of 1x sample dilution buffer was pipetted into blank wells, 100 µL of 1x sample dilution buffer was pipetted into maximum binding control wells. Standard, sample or control (100 µL each) was pipetted into the appropriate wells. HRP conjugate (100 µL) was pipetted into all wells except blank control wells, which wells were covered with adhesive strip and incubated for 2 hours. Following incubation, wells were washed manually three times with 400 µL wash buffer. After the final wash, the microplate was inverted and blotted dry on clean absorbent paper towels. After drying microplate, 200µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was pipetted into all wells, and incubated for 15-30 minutes at room temperature. Sulphuric acid (2N, 50 µL) was pipetted into all wells, mixed thoroughly before optical density was obtained within 30 minutes at a wavelength of 450nm (primary) and 570nm (reference). The concentration of 8-isoprostane was obtained from the standard curve (see appendix 9 for example standard curve). The intra and inter assay CV was 5.9% and 11.1% respectively.

2.2.14.3 Lipid profile

2.2.14.3.1 Plasma TAG concentration determination

The quantitative enzymatic TAG determination kit measured glycerol, true and total TAG concentration in plasma (Sigma-Aldrich, Dorset, UK). Prior to the assay, free glycerol reagent (powder) was reconstituted by adding 40 mL of deionised water, the solution was mixed thoroughly by inversion and protected from light using foil. TAG reagent (powder) was reconstituted by adding 10 mL of deionised water. The solution was mixed thoroughly by inversion and protected from light using foil. Spectrophotometer wavelength was set at 540nm and water was used as reference.

Following reagent preparation, 800 µL of free glycerol reagent was pipetted into each cuvette. Water, standard, sample and control (10 µL each) were pipetted into labelled cuvettes respectively and incubated for 5 minutes at 37°C. Initial absorbance (IA) of blank, standard, sample and control were read at 540nm versus water as reference. After reading, 200 µL of reconstituted TAG reagent was pipetted into each cuvette, mixed thoroughly by inversion and incubated for a further 5 minutes. Final absorbance (FA) was read after last incubation at 540 nm versus water as reference.

Concentrations of total TAG were calculated using the formula:

$$\frac{(FA_{\text{sample}} - FA_{\text{blank}})}{(FA_{\text{standard}} - FA_{\text{blank}})} \times \text{concentration of standard}$$

Intra and inter assay CV for TAG were 2.6% and 6.2% respectively

2.2.14.3.2 Plasma HDL-C concentration determination

Plasma HDL-C was measured using a quantitation kit (Sigma-Aldrich, Dorset, UK). Samples for HDL-C were first separated by adding equal volumes of precipitation buffer (100 µL) with sample (100 µL). The mixture was micro-centrifuged for 10 minutes at 2000 x g to ensure proper mixing, followed by incubation for 10 minutes. After incubation, tubes were further centrifuged for 10 minutes at room temperature, after this, the supernatant or HDL fraction was transferred to a new tube. Cholesterol standard solution (20 µL) was diluted with 140 µL cholesterol assay buffer to yield a 0.25 µg/µL standard from which serial dilutions were prepared. Cholesterol esterase

and enzyme mix were reconstituted separately by adding 220 µL of cholesterol assay buffer to each. The reaction mix contains 44 µL of cholesterol assay, 2 µL each of cholesterol probe, reconstituted cholesterol esterase and enzyme mix to yield a final volume of 50 µL.

Following sample and reagent preparation, sample (50 µL) and reaction mix (50 µL) were pipetted into each microplate well. This was thoroughly mixed using a horizontal plate shaker and incubated for 60 minutes at 37°C whilst protected from light. Optical density was read at 570nm. HDL-C concentrations were obtained from standard curve (see appendix 9 for example standard curve), obtained concentrations were multiplied by the corresponding dilution factor.

Intra and inter assay CV were 3.4% and 8.1% respectively.

2.2.14.3.3 Plasma Total cholesterol (TC) concentration determination

The enzymatic-colorimetric cholesterol liquid (Alpha Laboratories, Eastleigh, UK) was used to determine TC concentration in plasma. Prior to the assay, 3 mL of deionised water was added to calibrator (lyophilised serum) to make a solution from which serial dilutions were produced. After this, 3 µL of standard, control and sample were pipetted into appropriate sterilised wells followed by the addition of reagent 1 (300 µL). Following this, microplate was incubated for 10 minutes, after incubation, optical density was obtained at a wavelength of 505nm (primary) and 700nm (reference). TC concentrations were obtained from the standard curve (see appendix 11 for example standard curve). Intra and inter assay CV were 5.2% and 6.4% respectively.

2.2.14.3.4 Plasma LDL-C concentration determination

LDL-C concentration was determined using Friedewald formula (Friedewald, Levy, & Fredrickson, 1972).

Plasma LDL-C = Plasma TC – Plasma HDL-C – (TRG/2.2)

2.2.14.3.5 Plasma Non-High Density Lipoprotein-C (non-HDL-C) concentration determination

Non –HDL-C concentration was determined using the formula: Non-HDL-C = TC - HDL-C.

2.2.14.4 Renin angiotensin system

2.2.14.4.1 Plasma renin concentration determination

Renin concentration in plasma was determined using a solid-phase quantitative sandwich ELISA kit (R&D Systems Europe, Abingdon, UK) pre-coated with a monoclonal antibody specific for human renin. Prior to the assay, all samples were diluted two-fold (sample (75 μ L) and calibrator diluent (75 μ L). Wash buffer was mixed gently until the crystals dissolved, after mixing, 20 mL was diluted with 480 mL of deionised water to yield a final volume of 500 mL. Substrate solutions (colour A+B) were mixed together in equal volumes within 15 minutes of use and protected from light. Calibrator diluent was diluted 1:5 fold with deionised water, standard was reconstituted with 1.0 mL of deionised water to produce a stock solution, and was allowed to sit for 15 minutes before making serial dilutions.

Following sample and reagent preparation, 100 μ L of assay diluent was pipetted into each well. Standard, control, and sample (50 μ L each) were also pipetted into appropriate wells, which were covered with adhesive strip and incubated for 2 hours. After incubation, wells were decanted, washed 3 times manually with wash buffer (400 mL) and blotted dry against clean absorbent paper towels. Renin conjugate (200 μ L) was pipetted into each well, and incubated for 2 hours. Following incubation, wells were washed and blotted dry. After washing, 200 μ L substrate solution was pipetted into each well, covered with adhesive strip and foil to protect from light and incubated for 30 minutes. Stop solution (50 μ L) was pipetted into wells and the plate was tapped gently on bench top to ensure thorough mixing. Following thorough mixing, optical density was determined within 30 minutes at a wavelength of 450nm (primary) and 570nm (reference). Renin concentrations were obtained from a standard curve (see appendix 9 for example standard curve). Obtained concentrations were multiplied by the corresponding dilution factor. The intra and inter assay CV were 8.9 % and 5.3% respectively.

2.2.14.4.2 Plasma angiotensin II concentration determination

Plasma angiotensin II concentration was determined using a competitive enzyme immunoassay kit (Sigma-Aldrich, Dorset, UK). Assay diluent E (50 μ L) was added to centrifuged anti-angiotensin II detection antibody to prepare the detection

antibody concentrate. The solution was mixed thoroughly and further diluted 100-fold (50:4950 μ L) with assay diluent E to prepare the anti-angiotensin II antibody working solution. Biotinylated angiotensin II peptide (10 μ L) was added to assay diluent E (5 mL) to prepare 20 g/ mL biotinylated angiotensin II. Standard (8 μ L) was added to 20 pg/ mL biotinylated angiotensin II (792 μ L) to prepare angiotensin II stock solution from which serial dilutions were made.

Following reagent preparation, 100 μ L anti-target antibodies were pipetted into each well, which were covered with adhesive strips and incubated for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec) using a microplate shaker. Wells were washed 4 times with 300 μ L wash buffer. After the wash, wells were blotted dry against absorbent paper towels. Standard, control, blank and samples (100 μ L each) were pipetted into appropriate wells which were covered with adhesive strips and incubated for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec). Following incubation, 100 μ L of HRP-streptavidin solution was pipetted into each well and incubated for 45 minutes with gentle shaking at room temperature. After incubation, wells were washed 4 times with 300 μ L of wash buffer. After the final wash, wells were decanted and blotted dry against absorbent paper towels. TMB (100 μ L) was pipetted into each well and incubated for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec). Stop solution (50 μ L) was pipetted into each well and optical density was read immediately at 450 nm. Angiotensin II concentrations were obtained from the standard curve (see appendix 9 for example standard curve). Intra and inter assay CV were 9.5% and 8.9 % respectively.

2.2.14.5 Bone health markers

2.2.14.5.1 Total plasma 25(OH)D concentration determination

Plasma total 25(OH)D was measured using mini VIDAS® (BioMérieux, Hampshire, UK), a compact multi-parametric automated immuno-analyser based on the enzyme linked fluorescent assay principles. The 25(OH)D total assay kit contained a ready to use solid phase receptacle (SPR) and stabiliser strips coated with vitamin D. Plasma (100 μ L) was pipetted into each SPR well and inserted in machine. After 40 minutes run time, 25(OH)D concentration was obtained. The intra and inter assay CV were 2.0 % and 7.3 % respectively.

2.2.14.5.2 Plasma 25(OH)D concentration determination (ELISA).

The competitive binding solid phase direct sandwich ELISA kit (Calbiotech, Spring valley, USA) precoated with anti-vitamin D antibody, quantitatively determined 25(OH)D concentration in plasma. The ELISA kit was only used for plasma samples with a 25(OH)D concentration < 20.3 nmol/L which could not be detected by the automated analyser. Wash buffer (25 mL) was pipetted into 475 mL deionised water to yield a final volume of 500 mL. Prior to use, 1x working solution was diluted by adding 0.1 mL of the 51x vitamin D-biotin conjugate concentrate to 5 mL of assay diluent.

Following reagent preparation, 10 µL of standards, controls and samples were pipetted into appropriate wells. Biotinylated 25(OH)D (200 µL) was pipetted into each well, mixed for 20 seconds using a plate shaker set to 300 rpm, covered with an adhesive strip, and incubated for 90 minutes at room temperature. Wells were washed 3 times with 300 µL of wash buffer, decanted and blotted dry on absorbent paper towels. Enzyme conjugate (200 µL) was pipetted into each well, which were covered with adhesive strip and incubated for 30 minutes at room temperature. After incubation, 200 µL of TMB substrate was pipetted into each well and incubated in the dark for 30 minutes at room temperature. Stop solution (50 µL) was pipetted into each well, mixed on a shaker for 20-30 seconds. After mixing, optical density was determined within 10 minutes at a wavelength of 450nm. Concentrations of 25(OH)D were obtained from standard curve (see appendix 9 for example standard curve). Intra and inter assay CV were 11.3 and 3.6 % respectively.

2.2.14.5.3 Plasma PTH concentration determination

A solid phase direct sandwich ELISA kit (Calbiotech, Spring valley, USA) precoated with streptavidin, quantitatively determines PTH concentration in plasma. Prior to the assay, 25 mL of 1x wash buffer was added to 475 mL deionised water to yield a final volume of 500 mL. Stock enzyme conjugate (0.5 mL) was added to assay diluent (9.5 mL).

Following reagent preparation, standard, blank, control and samples (25 µL each) were pipetted into appropriate wells. Anti-PTH-biotin reagent (50 µL) and anti-PTH-HRP conjugates (50 µL) were pipetted into all wells respectively. The microplate

was covered with adhesive strip and incubated at room temperature on a plate shaker set at 500-600 rpm for 90 minutes.

Following incubation, wells were washed 4 times with 300 μ L wash buffer, decanted and blotted dry on clean absorbent paper towels. Following the wash step, 100 μ L of TMB substrate was pipetted into all wells, and incubated for 15 minutes at room temperature. Stop solution (50 μ L) was pipetted into each well and optical density was determined within 15 minutes at a wavelength of 450nm (primary) and 630nm (reference). PTH concentrations were obtained from a standard curve (see appendix 9 for example standard curve). Intra and inter assay CV were 4.7% and 2.6% respectively.

2.2.14.6 Inflammation

2.2.14.6.1 Plasma hs-CRP concentration determination

A solid phase direct sandwich ultra-sensitive ELISA kit (Calbiotech, Spring valley, USA) precoated with anti-CRP monoclonal antibody, quantitatively determined hs-C reactive protein concentration in plasma. Prior to the assay, samples and controls were diluted 1:100 fold by adding 5 μ L of sample to 495 μ L sample diluent. Wash buffer (25 mL) was added to 475 mL deionised water to yield a final volume of 500 mL.

Following reagent preparation, 10 μ L standard, blank, control and sample were pipetted into appropriate wells. Enzyme conjugate (100 μ L) was pipetted into each well, which were covered with adhesive strip, tapped gently on bench top to ensure thorough mixing and incubated for 1 hour at room temperature. Wells were washed three times with 300 μ L wash buffer, decanted and blotted dry on absorbent paper towels. TMB substrate (100 μ L) was pipetted into all wells, and incubated for 15 minutes at room temperature. Stop solution (50 μ L) was pipetted into each well, after which optical density was determined within 15 minutes at a wavelength of 450nm. Concentration of hs-CRP were obtained from standard curve (see appendix 9 for example standard curve), and obtained concentration was multiplied by the corresponding dilution factor. The intra and inter assay CV were 6.0% and 2.3% respectively.

2.2.14.7 Metabolic disease

2.2.14.7.1 Plasma insulin concentration determination

The solid phase direct sandwich ELISA kit (Calbiotech, Spring valley, USA) pre-coated with anti-insulin monoclonal antibody, quantitatively determined insulin concentration in plasma. Prior to experiment, 25 mL of wash buffer was added to 475 mL deionised water to yield a final volume of 500 mL. Stock enzyme conjugate (0.5 mL) was added to 9.5 mL assay diluent.

Following reagent preparation, 25 μ L standard, blank, control and sample were pipetted into appropriate wells. Enzyme conjugate (100 μ L) was pipetted into each well, which were covered with adhesive strip, mixed by tapping gently for 10 seconds on bench top and incubated for 60 minutes at room temperature. After incubation, wells were washed 3 times with 300 μ L wash buffer, wells were decanted and blotted dry on absorbent paper towels. TMB substrate (100 μ L) was pipetted into all wells, and incubated for 15 minutes at room temperature. After incubation, 50 μ L of stop solution was added to each well and optical density was determined within 15 minutes at a wavelength of 450nm. Sample concentrations were obtained from a standard curve (see appendix 9 for example standard curve) and obtained concentration was multiplied by the corresponding dilution factor. The intra and inter assay CV were 3.4% and 8.9% respectively.

2.2.14.7.2 Plasma glucose concentration determination

Fasting plasma glucose concentration was quantitatively determined using a colorimetric enzyme reagent kit (Alpha Laboratories, Eastleigh, UK). Prior to the assay, 1 vial of chromogen reagent containing mutarotase, glucose oxidase, peroxidase, 4-Aminoantipyrine and ascorbate oxidase was dissolved in 150 mL buffer. Following reagent preparation, 2 μ L of standard, blank, sample and control were pipetted into appropriate wells and 300 μ L of prepared chromogen reagent was pipetted into all wells and incubated for 5 minutes. Following incubation, optical density of glucose was obtained from a standard curve (see appendix 9 for example standard curve). The intra and inter assay CV was 3.0%. and 5.3% respectively

2.2.14.7.3 Insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) (Haffner, Kennedy, Gonzalez, Stern, & Miettinen, 1996), was used to evaluate insulin resistance and HOMA was calculated from the formula: $\text{HOMA-IR} = (\text{fasting glucose conc. (mmol/L)} \times \text{fasting insulin conc (mU/L)})/22.5$.

2.2.15 Statistical analysis

Data analysis was performed using IBM SPSS statistics software (IBM SPSS version 22, NYC, USA), and statistical significance was accepted at $P < 0.05$. For continuous variables including the arterial function and biochemical parameters, descriptive statistics were calculated and reported as mean \pm standard deviation (SD). Continuous variables were first assessed for normal distribution using Shapiro Wilks and homogeneity of variance was assessed by Levenes statistics. Difference in baseline characteristics were assessed using independent t- test, and a Mixed Model ANOVA was performed to assess changes between week 0, 4 and 8 for continuous variables including BMI, weight, SBP, DBP, MAP, HR, RT and non-HDL-C. Independent t- test was also used to assess continuous variables that were normally distributed with a homogenous variance between groups at either baseline, 4 or 8 weeks but not at all 3 time points such as in angiotensin II, LDL-C, TC, TAG and PWV(week 0 and 4), as well as PP (week 4 and 8) and PPao (week 0 and 8). Mann Whitney U-test was used to assess continuous variables that were not normally distributed between groups such as in 25(OH)D, PTH, renin, sE-selectin, 8-isoprostanes, HDL-C, hs-CRP, glucose, insulin, SBPao, HOMA-IR, WC and AIX (peripheral and central) (at all 3 time points) and Angiotensin II, LDL-C, TC, TAG and PWV (all at week 8). The assessment of PP, PPao and PWV at week 0, 4 and 8 respectively was performed using Mann Whitney U-test.

A paired students t-test was used to assess normally distributed repeated measures at week 0, 4 and 8 such as angiotensin II, LDL-C, TC, non-HDL-C, TAG at week 0 and 4), WC, PP, 25(OH)D, hsCRP and SBPao at week 4 and 8, PPao (week 8). Wilcoxon's t-test was used to assess non-normally distributed repeated measures at week 0, 4 and 8 such as glucose, PTH, renin, sE-selectin, insulin, HDL-C, AIX (peripheral and central). The continuous variables including WC, PP, 25(OH)D,

hsCRP and SBPao WC at week 0, PPao (week 0 and 4) and angiotensin II, LDL-C, TC, NHDL-C, TAG at week 8 were also assessed using Wilcoxon's t-test.

Association between changes in 25(OH)D at week 4 and 8 with corresponding changes in PWV, MAP, SBP, DBP, SBPao, PPao, HR, RT, TC, non-HDL-C, hs-CRP, sE-selectin, ang II, PTH, BMI, weight, as well as 8 week changes in HDL-C and PP and 4 week changes in AIX were all assessed using Pearson's correlation coefficient. In addition, the association of 4 and 8 week change in 25(OH)D with corresponding changes in TAG, HOMA-IR and insulin, 4 week change PP and HDL-C as well as 8 week change in AIX were determined using Spearman's correlation coefficient.

2.3 RESULTS

Of the 91 potential participants that were screened for eligibility, 78 were eligible, 23 out of the 78 did not attend clinic 2, and hence a total of 55 males were randomised to the intervention (28 in the vitamin D group and 27 in the placebo group). During the intervention 6 participants were lost to follow-up leaving 49 participants which include 7 Africans, 2 Asians and 40 Caucasians (25 in the vitamin D group and 24 in the placebo group) to complete the study (**Fig 2.3.1**). The rate of compliance was high, 90% in the vitamin D group and 87% in the placebo group. No adverse events were reported from taking the supplements at the end of the study.

2.3.1 Baseline characteristics

There were no significant differences in baseline characteristics between groups including age, body weight, BMI, waist circumference, HOMA plasma 25(OH)D, PTH, hs-CRP, 8-Isoprostanes, insulin, renin and angiotensin II concentrations except for the vitamin D group having a slightly higher mean (\pm SD) plasma glucose 5.23 ± 1.45 vs. 4.75 ± 0.82 mmol/L, ($P = 0.025$) compared to placebo (**Table 2.3.1**). There were also no significant differences in mean baseline plasma sE-selectin, and the haemodynamic parameters (**Table 2.3.2**), as well as baseline TC, non-HDL-C, HDL-C, LDL-C and TAG (**Table 2.3.3**).

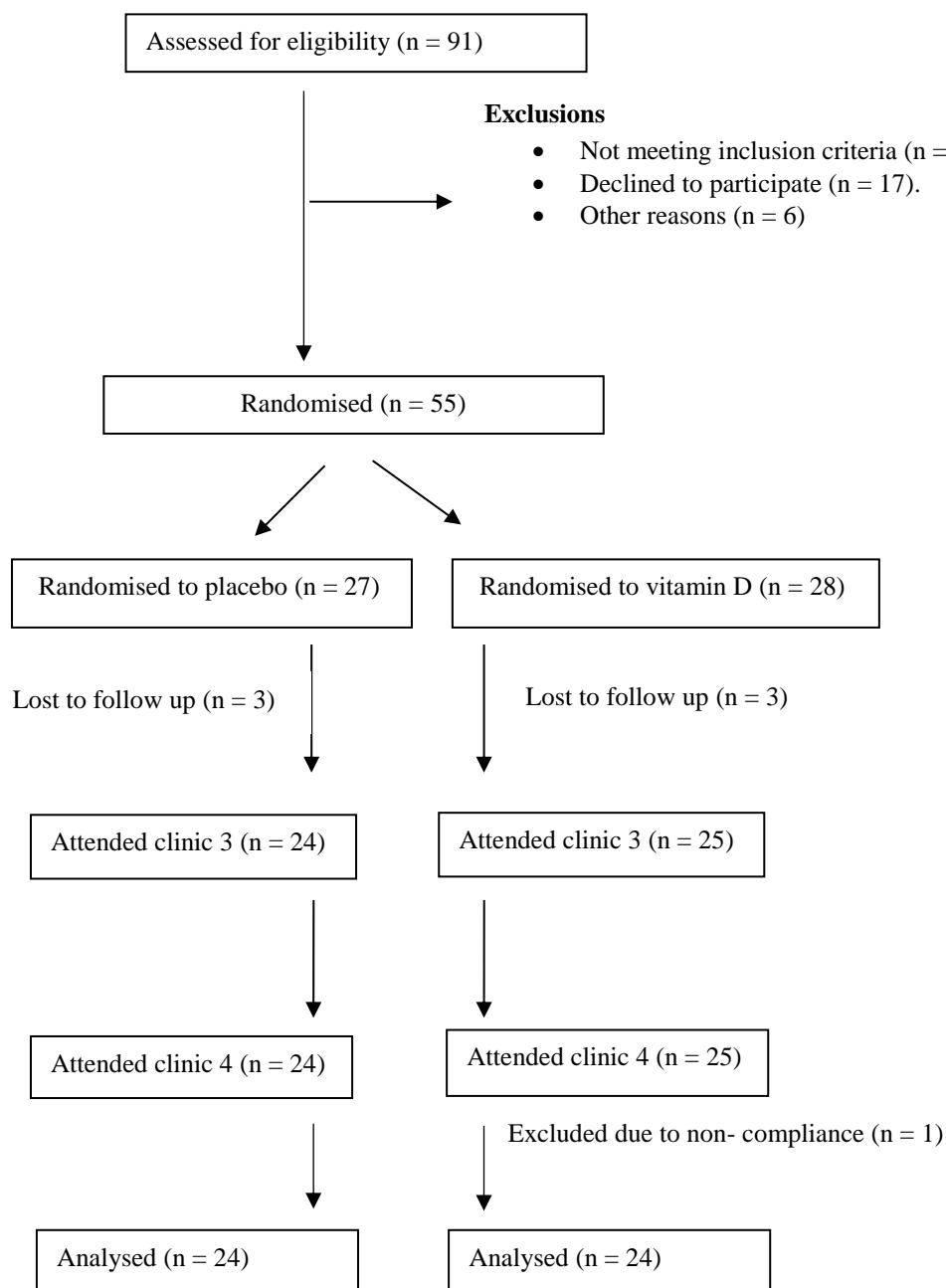


Fig.2.3.1. Study flow diagram of participants in the trial

Table 2.3.1 Baseline characteristics of study participants

Parameters	Vitamin D (n = 24)	Placebo (n = 24)	P	Normal ranges	References
Age (years)	35.9 ± 11.8	33.1 ± 12.2	0.248	-	
Body weight (kg)	92.4 ± 10.3	90.4 ± 19.3	0.378	-	
Body mass index (kg/m²)	29.9 ± 3.3	28.4 ± 2.6	0.071	18.5 - 24.9 kg/m ²	(Caballero, 2007)
Waist circumference (cm)	93.5 ± 22.1	91.3 ± 8.0	0.085	< 102 cm	(Bei-Fan, 2002)
Plasma 25(OH)D (nmol/L)	38.4 ± 15.9	43.9 ± 19.5	0.544	≥ 75 nmol/L	(Holick, 2007)
Plasma PTH (pmol/L)	4.3 ± 3.5	4.2 ± 2.8	0.917	1.1-6.8 pmol/L	(Lombardi et al., 2008)
Plasma insulin (pmol/L)	46.5 ± 27.7	42.1 ± 37.0	0.160	< 174 pmol/L	(Clare, Post, Bailey, Nestler, & Blackard, 1992)
HOMA-IR	1.6 ± 1.3	1.4 ± 1.6	0.068	1.7- 2.0	(Gayoso-Diz et al., 2013)
Plasma glucose (mmol/L)	5.2 ± 1.5	4.8 ± 0.8	0.025	< 5.6 mmol/L	(Tirosh et al., 2005)
Plasma 8-Isoprostanes (pg/ml)	11.2 ± 8.7	8.9 ± 5.7	0.449	35 ± 6 pg/ml	(Milne et al., 2007)
Plasma hs- CRP (mg/L)	2.9 ± 1.9	2.8 ± 2.1	0.480	< 1.0 mg/L	(Buckley, Fu, Freeman, Rogers, & Helfand, 2009)
Plasma renin (pg/mL)	639.8 ± 294	695.1 ± 410.7	0.716	201-1852 pg/ml	R&D systems
Plasma angiotensin II (pg/mL)	32.3 ± 9.6	31.8 ± 9.8	0.810	27 ± 4 pg/ml	(Arnold et al., 2013)

Values are presented as mean ± standard deviation

Mean baseline plasma 25(OH)D concentration in both groups was below the normal range of ≥ 75 nmol/L (Holick, 2007) and mean baseline plasma PTH in both groups was within the normal range of 1.1-6.8 pmol/L (Lombardi et al., 2008). Fasting mean plasma glucose concentration in both groups were within the normal range of < 5.55 mmol/L (Tirosh et al., 2005), fasting mean plasma insulin concentration in both groups were also within the normal range of < 174 pmol/L (Clare et al., 1992). Mean HOMA-IR in the present study ranged from 1.4-1.6 which were below the normal range of 1.7-2.0 (Gayoso-Diz et al., 2013). Mean baseline plasma 8-Isoprostanes concentrations in both groups were within the normal concentrations of 8-Isoprostanes in human plasma 35 ± 6 pg/mL (Milne et al., 2007). Mean baseline plasma renin concentrations of participants in both groups were also within the normal range of 201-1852 pg/ml, however, the mean baseline plasma angiotensin II concentrations of participants in both groups were comparable to the normal range of 27 ± 4 pg/mL (Arnold et al., 2013). Mean baseline plasma hs-CRP concentration in both groups were above the normal range < 1.0 mg/L (Buckley et al., 2009).

Participants' mean baseline SBP and DBP in both groups were less than the value for hypertension (>140 mmHg SBP and/or 90 mmHg DBP) (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006; Mancia et al., 2013). Mean baseline PWV in both groups was within normal reference of < 9.0 m/s (Tensiomed, 2016), however, brachial augmentation index normal reference are unknown. Central or aortic augmentation index at baseline for both group were within the normal reference $<33\%$ (Tensiomed, 2016). Mean baseline aortic central systolic blood pressure for both group were well within the normal range of < 140 mmHg (Tensiomed, 2016). Mean baseline references for aortic central pulse pressure for both groups were well within the normal values of < 50 mmHg (Tensiomed, 2016). Mean baseline return time for both groups were within the normal reference of > 124 m/s (Tensiomed, 2016) and mean baseline values for heart rate for both groups also were within the normal resting heart rate of 40-100 bpm (Lin et al., 2010). Although the normal range for brachial pulse pressure is not known, Lokaj et al., has suggested values < 55 mmHg as normal and participants fell within this normal range (Lokaj et al., 2011). Mean arterial pressure was within the normal range of 70 -105 mmHg (Hohn et al., 2013). Mean baseline plasma concentrations of sE-selectin are not comparable with the normal, as the normal concentration for sE-selectin is undefined (**Table 2.3.2**).

Table 2.3.2 Baseline markers of endothelial function, blood pressure and arterial stiffness indices

Parameters	Vitamin D (n = 24)	Placebo (n = 24)	<i>P</i>	Normal ranges	References
SBP (mmHg)	128.7 ± 11.1	131.2 ± 12.8	0.730	90-139 mmHg	(Mancia et al., 2013)
DBP (mmHg)	77.0 ± 9.7	78.0 ± 12.9	0.797	60-89 mmHg	(Mancia et al., 2013)
PP (mmHg)	53.6 ± 9.2	53.8 ± 9.2	0.846	Not known	-
MAP (mmHg)	95.1 ± 9.1	95.9 ± 12.0	0.796	70-105 mmHg	(Hohn et al., 2013)
PWV (m/s)	6.5 ± 1.1	6.5 ± 1.1	0.802	< 9.0m/s	(Tensiomed, 2016)
AIx (%)	-48.6 ± 25.9	-51.5 ± 22.2	0.953	Not known	-
AIxao (%)	12.6 ± 13.4	12.7 ± 13.6	0.940	<33%	(Tensiomed, 2016)
SBPao (mmHg)	118.5 ± 12.9	119.5 ± 16.9	0.873	< 140 mmHg	(Tensiomed, 2016)
PPao (mmHg)	41.6 ± 8.8	41.3 ± 8.7	0.927	< 50 mmHg	(Tensiomed, 2016)
Heart rate (bpm)	62.3 ± 12.3	60.9 ± 10.8	0.635	40-100	(Lin et al., 2010)
Return time (m/s)	153.3 ± 24.4	157.6 ± 24.9	0.443	> 124 m/s	(Tensiomed, 2016)
Plasma sE-selectin (ng/mL)	57.9 ± 31.1	57.5 ± 18.9	0.312	Not known	-

Values are presented as mean ± standard deviation

Table 2.3.3. Baseline lipid profile markers

Parameters	Vitamin D (n = 24)	Placebo (n = 24)	<i>P</i>	Normal ranges	References
Plasma TC (mmol/L)	6.5 ± 1.5	6.4 ± 1.6	0.758	< 5.2 mmol/L	(Glasziou, Irwig, Heritier, Simes, & Tonkin, 2008)
Plasma TAG (mmol/L)	1.7 ± 0.4	1.7 ± 0.3	0.665	< 1.7 mmol/L	(Alberti, Zimmet, & Shaw, 2006).
Non- HDL-C (mmol/L)	6.3 ± 1.8	6.2 ± 2.2	0.893	< 3.4 mmol/L	(Blaha, Blumenthal, Brinton, Jacobson, & Cholesterol, 2008).
Plasma HDL-C (mmol/L)	0.6 ± 0.1	0.7 ± 0.2	0.814	1.0-1.3 mmol/L in men	(Glasziou et al., 2008).
LDL -C (mmol/L)	5.2 ± 1.5	5.0 ± 1.6	0.719	< 2.6 mmol/L	(Glasziou et al., 2008).

Values are presented as mean ± standard deviation

Mean baseline plasma HDL-C concentrations for both groups were below the normal range of 1.0-1.3 mmol/L for men (Glasziou et al., 2008), and mean baseline LDL-C mean concentrations for both groups were above the normal range of < 2.6 mmol/L (Glasziou et al., 2008). Mean baseline plasma TC concentrations for both groups were above the normal range of < 5.2 mmol/L (Glasziou et al., 2008). Mean plasma non-HDL-C concentration for both groups was also above the normal range of < 3.4 mmol/L concentration ideal for individuals at risk of CVD (Blaha et al., 2008). However, mean plasma TAG concentrations for both groups were borderline, but not < 1.7 mmol/L (Alberti et al., 2006).

2.3.2 Baseline dietary intake of energy and nutrients among treatment group.

Based on reported consumption from the completed 3-day food diary at weeks 0 and 4 (n = 42), no significant difference was observed between the intervention and placebo group from mean dietary intake of energy, carbohydrate protein, fats and vitamin D (Table 2.3.4).

Table 2.3.4. Baseline dietary energy and nutrients intake among treatment group.

Daily mean nutrient intake	Vitamin D (n = 24)	Placebo (n = 24)	<i>P</i>
Energy (kcal/day)	1853.9 ± 500.8	2141.7 ± 663.8	0.194
Energy (MJ/day)	7.48 ± 2.2	8.79 ± 2.9	0.110
Fat (g/day)	68.4 ± 14.2	82. ± 27.4	0.062
Protein (g/day)	83.9 ± 24.5	106.9 ± 56.9	0.147
Carbohydrate (g/day)	219.4 ± 67.5	247.8 ± 85.1	0.251
Vitamin D (µg/day)	1.6 ± 1.2	3.1 ± 3.1	0.060

Values are presented as mean ± standard deviation

Table 2.3.5. Baseline dietary intake of energy and nutrients compared to the DRV and UK population

Energy and Nutrients	Males (n = 42)	DRV	NDNS
	Mean \pm SD		Mean \pm SD
Energy (MJ/day)	8.23 \pm 2.6	-	8.86 \pm 2.36 MJ/day
Protein (g/day)	97.1 \pm 46.9	55.5	84.6 \pm 17.6 g/day
% energy as protein	21.6%	15%	17.2%
Carbohydrates(g/day)	235.6 \pm 78.4	-	257 \pm 76 g/day
% energy as CHO	45.3%	50%	48.5%
Fat (g/day)	76.2 \pm 23.5	-	76.3 \pm 5.5 g/day
% Energy as fat	35%	35%	34.2%
Vitamin D (μg/day)	2.5 \pm 2.6	10	3.1 \pm 2.4

The mean dietary energy intake in MJ/day was comparable to the values reported for the corresponding age group in the recent National Dietary and Nutrition Survey (Public Health England, 2016). The mean dietary carbohydrate and vitamin D intake were lower than reported for the corresponding age group in the recent National Dietary and Nutrition Survey (Public Health England, 2016). The mean dietary protein and fat intake were above and within the reported values respectively for the corresponding age group in the recent National Dietary and Nutrition Survey (Public Health England, 2016).

2.3.3. Baseline plasma 25(OH)D concentrations

At baseline, 22.2 % (n = 12) of participants had plasma 25(OH)D concentrations < 25nmol/L, 35.2 % (n = 19) had plasma 25(OH)D concentrations < 30nmol/L and 64.8% (n = 35) of participants had plasma 25(OH)D concentrations < 50nmol/L independent of treatment group (**Fig 2.3.2**). No significant associations were found

between the stratified baseline plasma 25(OH)D concentrations and the cardiometabolic markers measured.

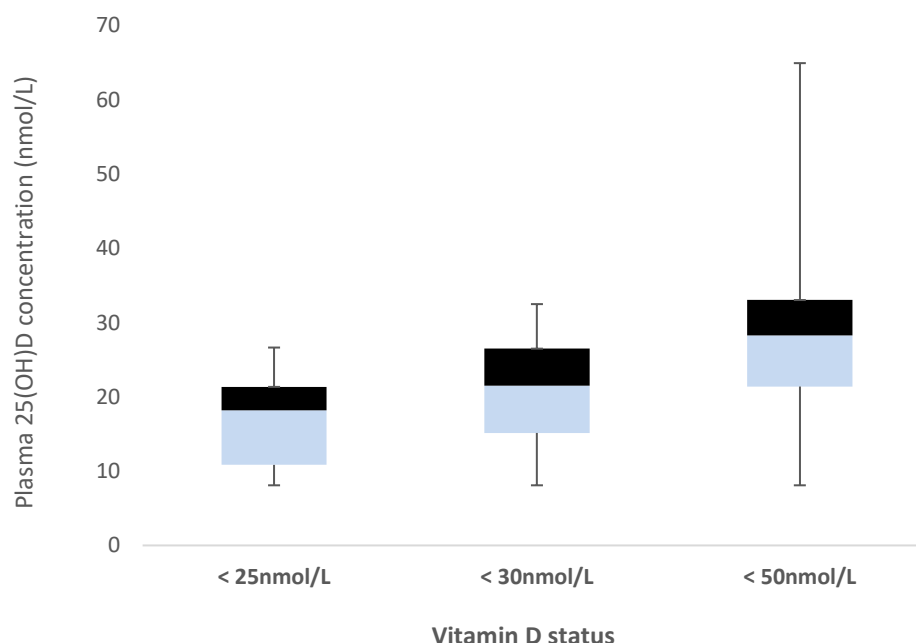


Fig 2.3.2 Box plot of the prevalence of low plasma 25(OH)D concentrations

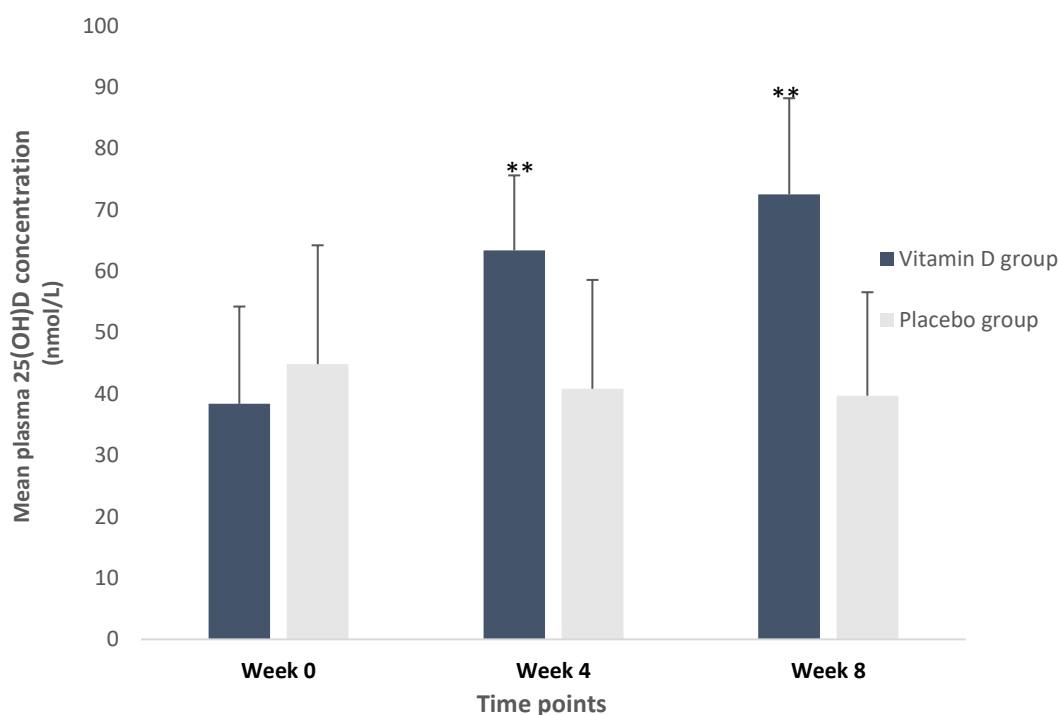
2.3.4. Effect of supplementation on plasma 25(OH)D and PTH concentrations

The administration of 5000 IU (125 μ g) vitamin D₃ resulted in a significant increase in plasma 25(OH)D concentrations compared to placebo. After 4 weeks intervention, mean (\pm SD) plasma 25(OH)D concentration increased by $+25.76 \pm 3.63$ nmol/L in the vitamin D group vs. -4.02 ± 2.09 nmol/L in those taking placebo. After 8 weeks of intervention, plasma mean (\pm SD) 25(OH)D concentrations increased by $+34.12 \pm 0.18$ in the vitamin D group vs. -5.14 ± 1.62 ($P < 0.001$) in the placebo group (**Fig.2.3.3**). There was no significant difference in change in plasma PTH concentrations at 4 and 8 weeks between the groups (**Table 2.3.6**). Vitamin D₃ supplementation increased plasma 25(OH)D concentrations to ≥ 75 nmol/L in 45.8 % (11/24) of participants in the vitamin D group, whereas no participants (0/24) in the placebo group achieved plasma 25(OH)D concentrations ≥ 75 nmol/L after 8 weeks of intervention.

Table 2.3.6. Effect of vitamin D supplementation on plasma 25(OH)D and PTH concentrations

Parameters	Time points	Vitamin D (n = 24)	Placebo (n = 24)	P
Plasma 25(OH)D (nmol/L)	0 vs. 4wks	+ 25.8 ± 3.6	- 4.0 ± 2.1	< 0.001
	0 vs. 8wks	+ 34.1 ± 0.2	- 5.1 ± 1.6	< 0.001
Plasma PTH (pmol/L)	0 vs. 4wks	+ 0.2 ± 0.0	+ 0.2 ± 0.0	0.893
	0 vs. 8wks	- 0.6 ± 0.5	+ 0.7 ± 0.3	0.112

Values are presented as mean ± standard deviation



****P < 0.001** represents difference at each time points between vitamin D and placebo group.

Fig.2.3.3. Effect of vitamin D supplementation on plasma 25(OH)D concentrations (± SD) at week 0, 4 and 8 weeks.

2.3.5 Effect of vitamin D supplementation on plasma sE-selectin concentrations and haemodynamic measures

Vitamin D₃ supplementation significantly decreased mean (\pm SD) brachial pulse pressure by -4.2 ± 5.3 vs. -0.3 ± 2.8 mmHg, ($P = 0.011$) at 4 weeks and decreased by -2.9 ± 3.4 vs. $+0.3 \pm 2.8$ mmHg, ($P = 0.027$) at 8 weeks in the vitamin D compared to placebo group (**Table 2.3.7**). No significant changes were observed in plasma sE-selectin concentration (**Fig.2.3.4.**), PWV (**Fig.2.3.5.**), AIx and the other arterial stiffness indices in both treatment groups at 4 and 8 weeks despite increased 25(OH)D concentrations in vitamin D group. However, within groups, there was a significant mean decrease of -21.8 and -32.9 ng/mL ($P < 0.001$) in plasma sE-selectin concentrations between weeks 4 and 0 in the vitamin D and placebo group respectively. Similarly, there was also a significant mean decrease of -18.8 and -32.4 ng/mL ($P < 0.001$) in plasma sE-selectin concentrations between weeks 8 and 0 in the vitamin D and placebo group respectively.

Table 2.3.7. Effect of supplementation on plasma sE-selectin concentrations and haemodynamic measures

Parameters	Time points	Vitamin D (n = 24)	Placebo (n = 24)	P
SBP (mmHg)	0 vs. 4wks	-2.0 ± 0.8	2.9 ± 0.1	0.096
	0 vs. 8wks	-0.9 ± 0.1	4.3 ± 0.2	0.099
DBP (mmHg)	0 vs. 4wks	-1.0 ± 0.9	1.3 ± 0.1	0.570
	0 vs. 8wks	0.0 ± 0.7	2.1 ± 0.5	0.522
PWV (m/s)	0 vs. 4wks	-0.1 ± 0.1	- 0.2 ± 0.2	0.581
	0 vs. 8wks	-0.1 ± 0.2	- 0.2 ± 0.1	0.423
PP (mmHg)	0 vs. 4wks	-4.2 ± 5.3	-0.3 ± 2.8	0.011*
	0 vs. 8wks	-2.9 ± 3.4	1.6 ± 0.5	0.027*
MAP (mmHg)	0 vs. 4wks	-1.7 ± 0.9	0.1 ± 0.4	0.796
	0 vs. 8wks	-0.9 ± 0.9	1.3 ± 0.7	0.413
Aix (Brachial) (%)	0 vs. 4wks	5.8 ± 1.1	6.0 ± 9.1	0.631
	0 vs. 8wks	15.4 ± 13.3	6.1 ± 8.9	0.940
Aix (Aortic) (%)	0 vs. 4wks	3.5 ± 0.5	-1.22 ± 4.0	0.682
	0 vs. 8wks	3.1 ± 0.7	-0.2 ± 3.2	0.705
SBPao (mmHg)	0 vs. 4wks	-1.6 ± 0.3	-0.1 ± 2.1	0.484
	0 vs. 8wks	-0.7 ± 0.2	1.9 ± 1.8	0.347
PPao (mmHg)	0 vs. 4wks	-1.1 ± 1.3	0.1 ± 2.1	0.645
	0 vs. 8wks	-0.7 ± 1.5	1.7 ± 1.8	0.280
HR (bpm)	0 vs. 4wks	-1.1 ± 0.6	1.1 ± 0.8	0.518
	0 vs. 8wks	-0.6 ± 0.6	0.0 ± 0.2	0.904
RT (m/s)	0 vs. 4wks	0.6 ± 2.4	5.3 ± 2.7	0.339
	0 vs. 8wks	1.1 ± 3.7	1.9 ± 2.4	0.715
Soluble E-selectin (ng/mL)	0 vs. 4wks	-13.7 ± 1.7	-18.3 ± 1.3	0.915
	0 vs. 8wks	-10.8 ± 1.4	-17.1 ± 2.9	0.733

Values are presented as mean ± standard deviation

* $P < 0.05$

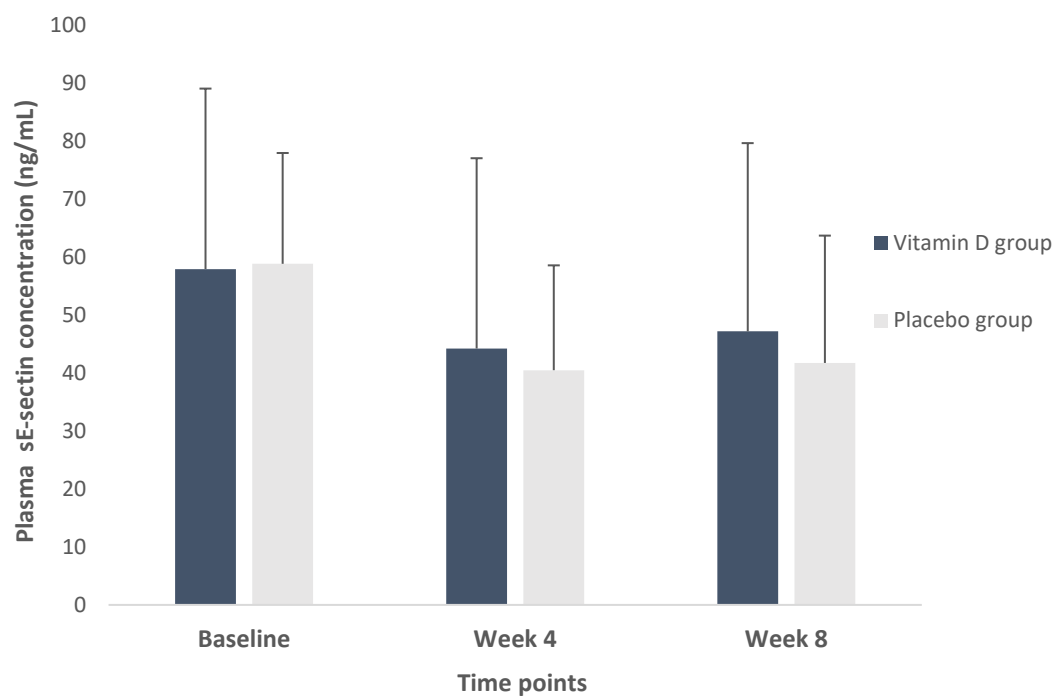


Fig. 2.3.4. Effect of vitamin D supplementation on plasma mean (\pm SD) sE-selectin concentration

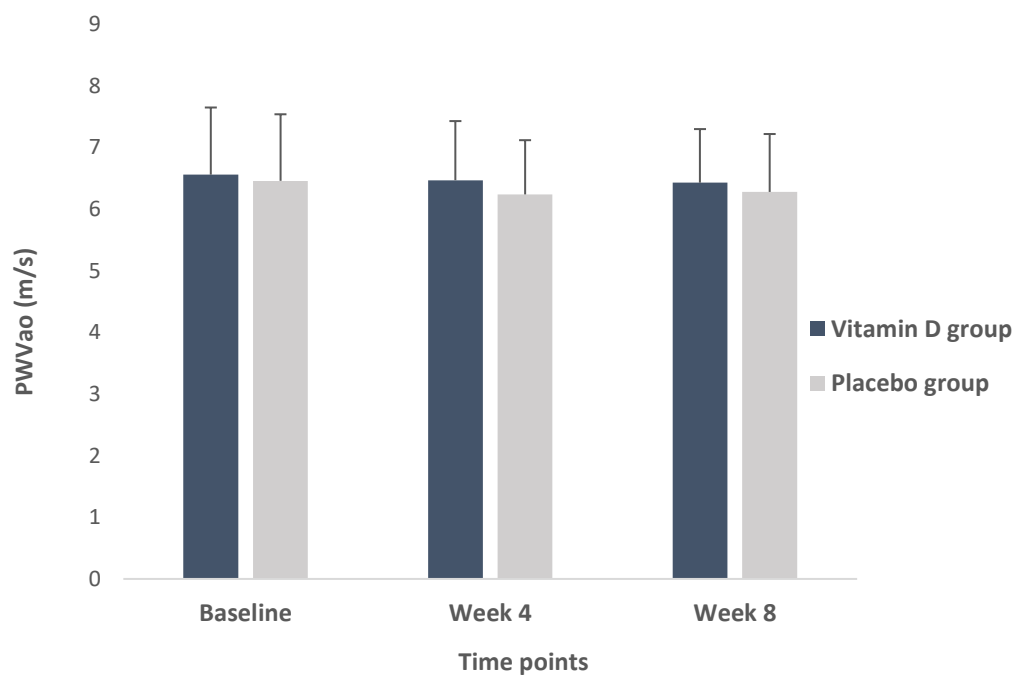


Fig. 2.3.5. Effect of vitamin D supplementation on mean (\pm SD) PWV

2.3.6. Effect of vitamin D supplementation on plasma hs-CRP, 8 -Isoprostanes, renin and angiotensin II concentrations

There were no significant changes in plasma renin, angiotensin II, hs-CRP and 8-isoprostanes concentrations between vitamin D and placebo group at 4 and 8 weeks despite improved vitamin D status (**Table 2.3.8**). However, within groups, there was a significant mean decrease of -133.46 and -261.26 pg/mL ($P < 0.001$) in plasma renin concentrations between weeks 8 and 0 in the vitamin D and placebo group respectively.

Table 2.3.8. Effect of vitamin D supplementation on plasma hs-CRP, 8-Isoprostanes, renin and angiotensin II concentrations

Parameters		Vitamin D (n = 24)	Placebo (n = 24)	<i>P</i>
Plasma hs-CRP (mg/L)	0 vs. 4wks	-0.5 ± 3.4	0.3 ± 0.03	0.678
	0 vs. 8wks	-0.2 ± 3.3	0.2 ± 0.02	0.264
Plasma 8-Isoprostanes (pg/ml)	0 vs. 4wks	5.3 ± 2.4	6.9 ± 6.8	0.653
	0 vs. 8wks	3.4 ± 2.8	7.5 ± 8.5	0.222
Plasma Renin (pg/mL)	0 vs. 4wks	-79.9 ± 41.8	-153.2 ± 167.4	0.781
	0 vs. 8wks	-96.3 ± 49.5	-223.5 ± 224.2	0.610
Plasma angiotensin II (pg/mL)	0 vs. 4wks	-1.3 ± 2.9	0.9 ± 2.7	0.163
	0 vs. 8wks	-1.5 ± 2.1	-3.9 ± 2.5	0.390

Values are presented as mean ± standard deviation

2.3.7. Effect of vitamin D supplementation on metabolic profile biomarkers

There was a significant decrease in change in plasma glucose concentration of -0.3 vs. 0.2 in the placebo but not in vitamin D group at 4 weeks, however, this decrease did not persist at 8 weeks (**Table 2.3.9**).

Table 2.3.9. Effect of vitamin D supplementation on metabolic profile markers

Parameters		Vitamin D (n = 24)	Placebo (n = 24)	P
Plasma Insulin (pmol/L)	0 vs. 4wks	-2.9 ± 0.8	6.2 ± 5.4	0.779
	0 vs. 8wks	-0.5 ± 10.4	3.5 ± 2.9	0.897
HOMA-IR	0 vs. 4wks	0.0 ± 0.1	0.0 ± 0.7	0.635
	0 vs. 8wks	0.1 ± 0.8	0.0 ± 0.3	0.680
Plasma glucose (mmol/L)	0 vs. 4wks	0.2 ± 0.1	-0.3 ± 0.1	0.011
	0 vs. 8wks	0.1 ± 0.6	-0.0 ± 0.3	0.209

Values are presented as mean ± standard deviation

2.3.8. Effect of vitamin D supplementation on lipid profile markers

In the present study, there were no significant changes between groups in plasma concentrations of the lipid profile markers measured (Table 2.3.10).

Table 2.3.10. Effect of vitamin D supplementation on lipid profile markers

Parameters		Vitamin D (n = 24)	Placebo (n = 24)	P
Plasma TC (mmol/L)	0 vs. 4wks	0.6 ± 0.3	0.4 ± 0.4	0.787
	0 vs. 8wks	1.3 ± 0.2	0.9 ± 0.1	0.216
Plasma TAG (mmol/L)	0 vs. 4wks	-0.1 ± 0.0	-0.1 ± 0.0	0.607
	0 vs. 8wks	0.0 ± 0.1	-0.1 ± 0.1	0.445
Non- HDL-C (mmol/L)	0 vs. 4wks	-0.7 ± 0.7	-0.6 ± 0.4	0.892
	0 vs. 8wks	0.1 ± 0.9	-0.0 ± 0.7	0.740
Plasma HDL-C (mmol/L)	0 vs. 4wks	0.0 ± 0.0	-0.1 ± 0.1	0.317
	0 vs. 8wks	0.1 ± 0.0	-0.1 ± 0.1	0.250
LDL-C (mmol/L)	0 vs. 4wks	0.5 ± 0.0	0.5 ± 0.0	0.807
	0 vs. 8wks	0.2 ± 0.9	0.0 ± 0.6	0.416

Values are presented as mean ± standard deviation

2.3.9. Effect of vitamin D supplementation on anthropometric measures

There were no significant changes in weight, WC and BMI between vitamin D and placebo groups after 4 and 8 week intervention despite improved vitamin D status (Table 2.3.11).

Table 2.3.11. Effect of vitamin D supplementation on anthropometric measures

Parameters		Vitamin D (n = 24)	Placebo (n = 24)	P
Body weight (kg)	0 vs. 4wks	-0.5 ± 0.4	- 0.8 ± 0.0	0.836
	0 vs. 8wks	-0.2 ± 0.8	0.3 ± 0.3	0.468
BMI (kg/m ²)	0 vs. 4wks	0.1 ± 0.2	0.2 ± 0.3	0.095
	0 vs. 8wks	0.2 ± 0.3	0.1 ± 0.2	0.068
Waist circumference (cm)	0 vs. 4wks	3.6 ± 10.9	0.5 ± 0.8	0.138
	0 vs. 8wks	3.7 ± 12.7	1.0 ± 1.4	0.098

Values are presented as mean ± standard deviation

2.3.10. Differences in haemodynamic measures between overweight and obese participants

A significant difference was observed in mean (±SD) SBP and DBP between overweight and obese participants at week 4. SBP and DBP was lower in the overweight (n = 14) compared to the obese (n = 10) group (123.1 ± 9.8 vs. 131.8 ± 9.2 mmHg, $P = 0.037$) (Fig.2.3.6) and (71.5 ± 8.1 vs. 82.5 ± 9.2 mmHg, $P = 0.008$) respectively (Fig.2.3.7). A significant difference was observed in mean (±SD) MAP between the overweight and obese group at week 4, MAP was lower in the overweight compared to the obese group (88.8 ± 8.2 vs. 99.7 ± 9.7 mmHg, $P = 0.008$). However, no significant difference was observed in SBP, DBP and MAP at week 0 and 8.

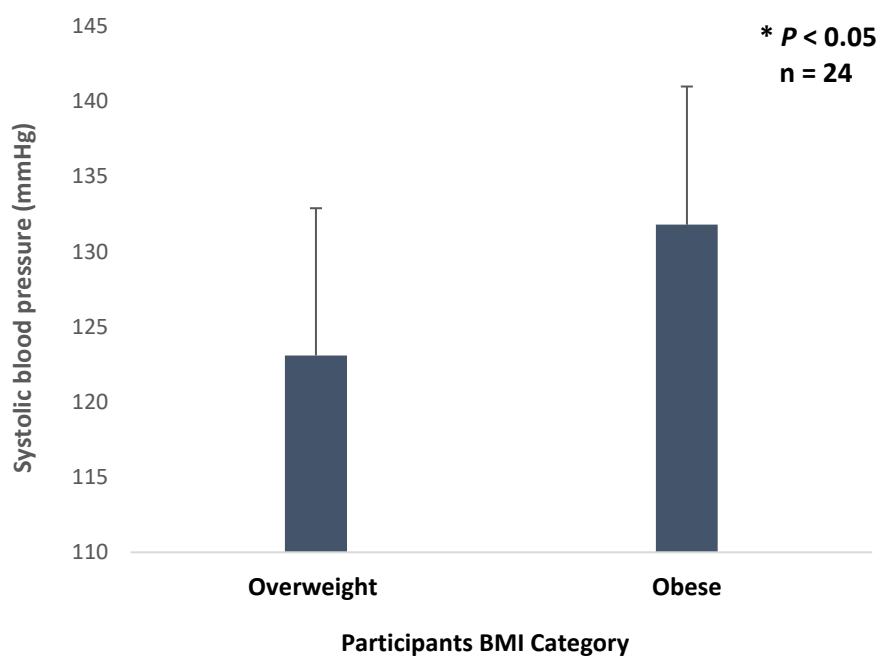


Fig.2.3.6. Mean (\pm SD) of SBP between overweight and obese participants in the vitamin D group at week 4

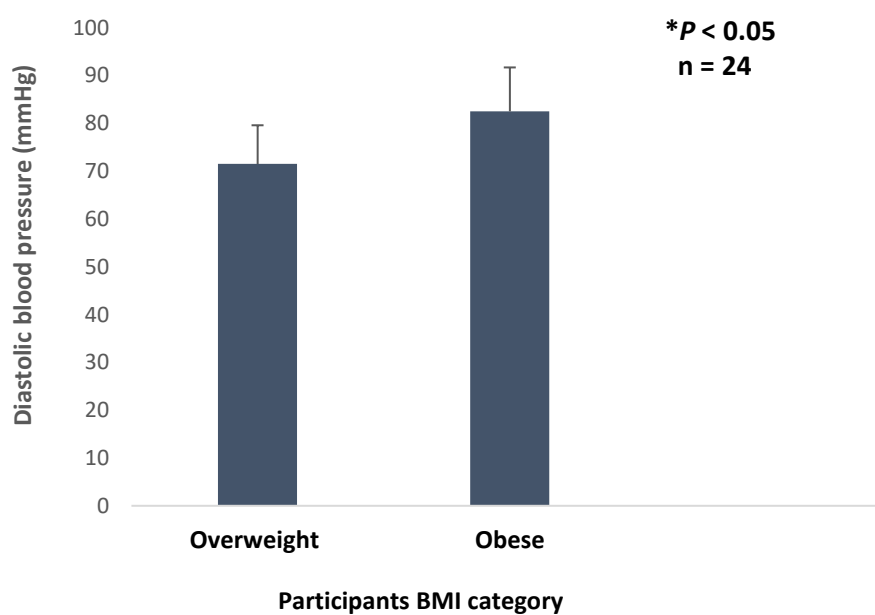


Fig.2.3.7. Mean (\pm SD) of DBP between overweight and obese participants in the vitamin D group at week 4

2.3.11. Associations between plasma 25(OH)D concentrations and cardiometabolic markers

Baseline plasma 25(OH)D concentration of all participants ($n = 54$) was inversely associated with plasma insulin ($r = -0.307$, $P = 0.024$) (Fig 2.3.8A) and HOMA-IR ($r = -0.276$, $P = 0.043$) (Fig 2.3.8B) irrespective of treatment group. Change in plasma 25(OH)D concentrations at week 4 was inversely associated with change in plasma glucose ($r = -0.408$, $P = 0.048$, $n = 24$) (Fig.2.3.9A), with baseline 25(OH)D concentrations ($r = -0.714$, $P < 0.001$) (Fig.2.3.9B), with change in aortic central pulse pressure ($r = -0.517$, $P = 0.010$, $n = 24$) (Fig. 2.3.9C) and change in HDL-C ($r = -0.459$, $P = 0.024$) (Fig. 2.3.9D).

Change in plasma 25(OH)D concentration at week 8 was inversely associated with baseline 25(OH)D concentrations ($r = -0.603$, $P = 0.002$, $n = 24$) (**Fig.2.3.10A**), change in plasma glucose concentration ($r = -0.513$, $P = 0.010$, $n = 24$) (**Fig.2.3.10B**). Change in plasma 25(OH)D concentrations at week 8 was inversely associated with changes in brachial augmentation index ($r = -0.446$, $P = 0.029$, $n = 24$) (**Fig. 2.3.10C**).

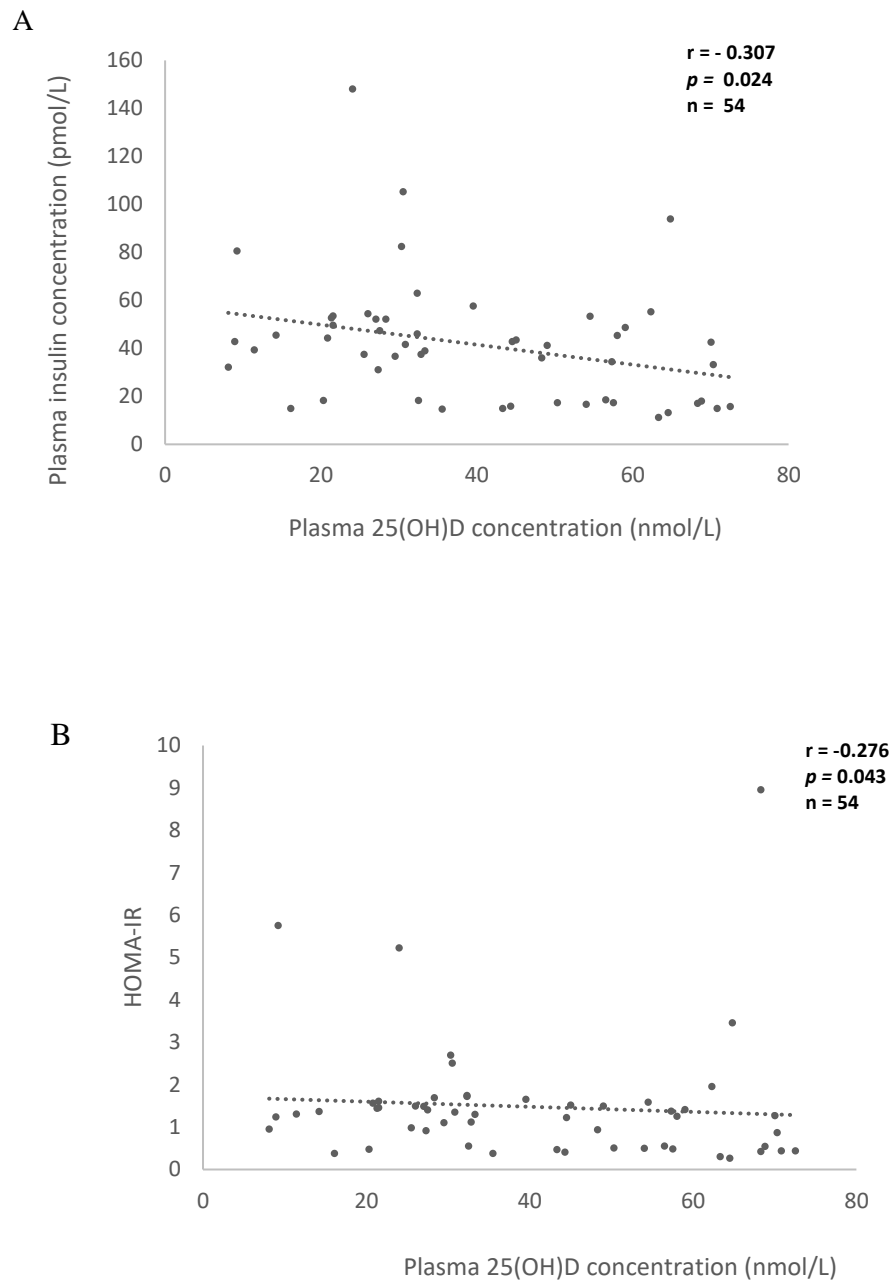


Fig 2.3.8 Baseline associations between plasma 25(OH)D concentrations with plasma insulin concentrations (A) and HOMA-IR (B)

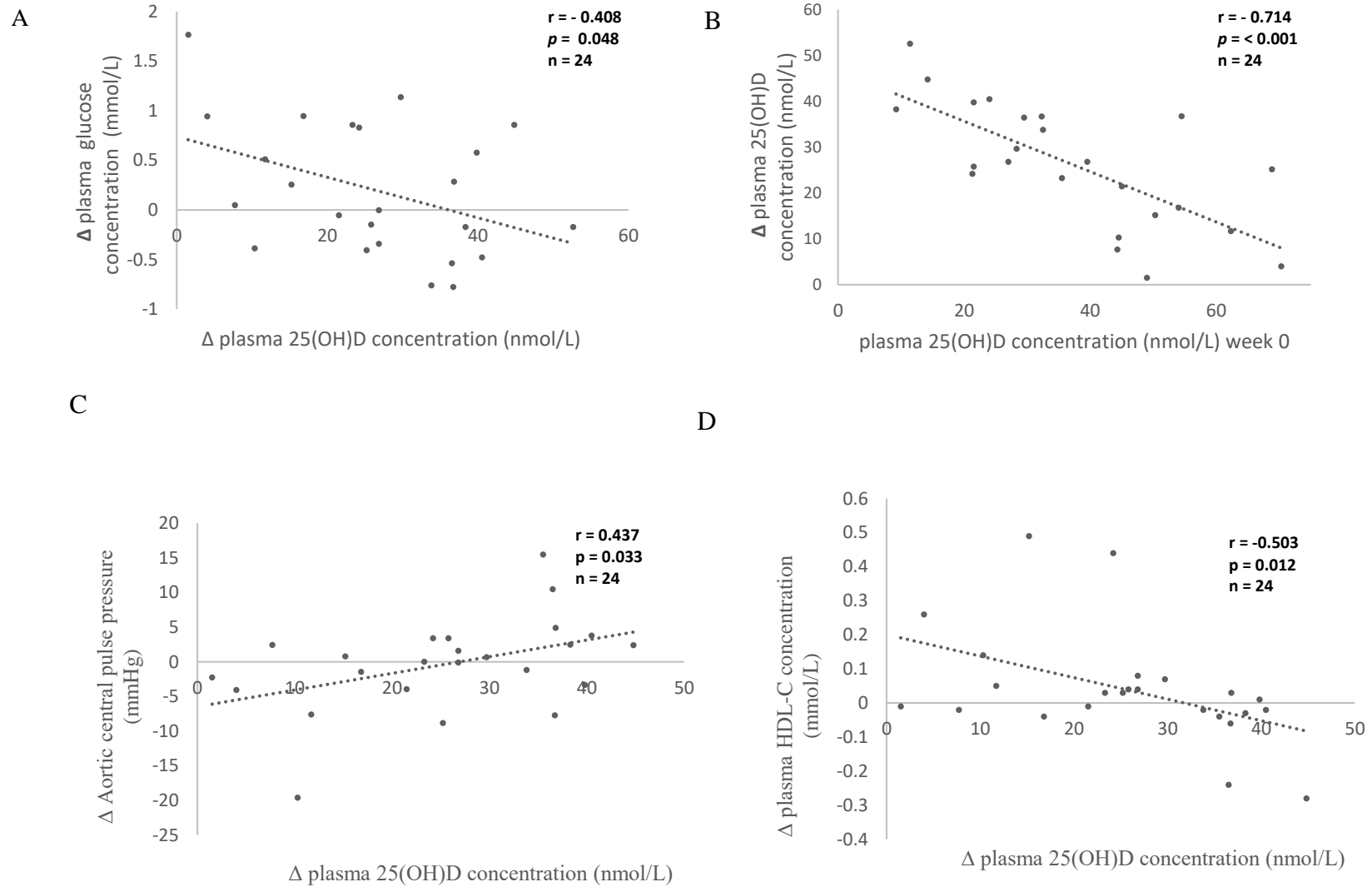


Fig.2.3.9 Associations between Δ plasma 25(OH)D concentration and (A) Δ plasma glucose concentration, (B) baseline plasma 25(OH)D concentration, (C) Δ aortic central pulse pressure and (D) Δ plasma HDL-C concentration at 4 weeks

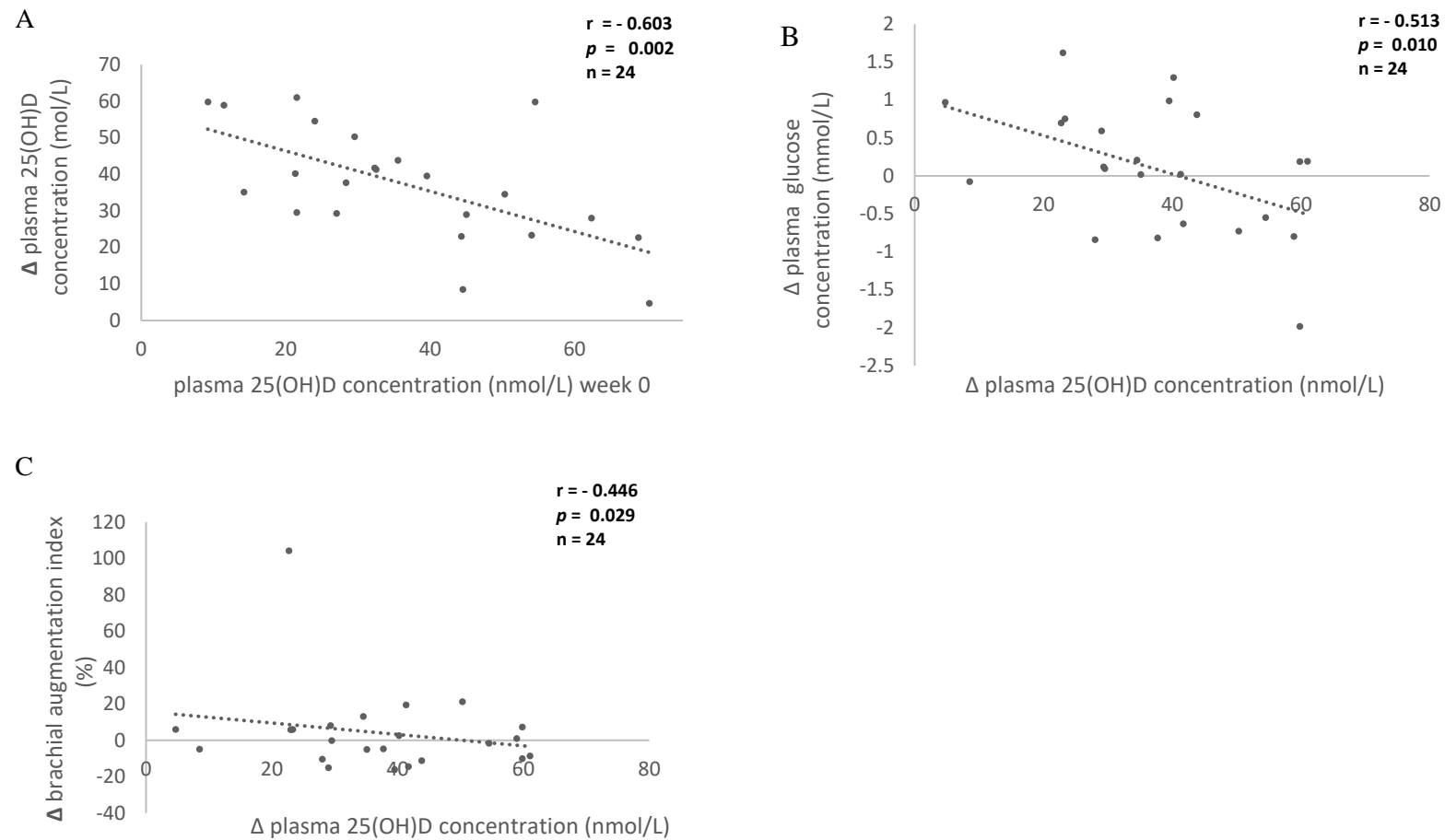


Fig.2.3.10 Associations between 8 week change in plasma 25(OH)D with baseline plasma 25(OH)D (A), change in plasma glucose (B) and change in brachial augmentation index (C)

2.4 DISCUSSION

To our knowledge, this is the first RCT investigating the effect of daily 5,000IU (125µg) vitamin D₃ for 8 weeks on a broad range of cardiometabolic risk markers in overweight and obese adult males. Although all study participants had suboptimal plasma 25(OH)D concentrations at baseline, 22.2 % (n = 12), 35.2 % (n = 19) and 64.8% (n = 35) of participants had plasma 25(OH)D concentrations < 25nmol/L, < 30nmol/L and < 50nmol/L respectively, independent of treatment group. No significant associations were found between the stratified baseline plasma 25(OH)D concentrations and the cardiometabolic biomarkers measured.

This RCT showed that 8 weeks of 5,000IU (125µg) vitamin D₃ improved mean (±SD) vitamin D concentration significantly from baseline by +25.8 ± 3.6 nmol/L, (*P* <0.001) at 4 weeks and + 34.1 nmol/L, (*P* <0.001) post intervention in the vitamin D group. The present study did not demonstrate a significant effect of vitamin D supplementation on the cardiometabolic markers including endothelial function, arterial stiffness, blood pressure and RAS, lipid profile, oxidative stress, and inflammation and glucose homeostasis parameters between treatment groups. However, beneficial effects were observed in brachial pulse pressure at 4 and 8 weeks in the vitamin D group, mean (±SD) brachial pulse pressure significantly decreased from baseline and 4 weeks, -4.2 ± 5.3 vs. -0.3 ± 2.8 mmHg, (*P* = 0.011) and from baseline and 8 weeks, -2.9 ± 3.4 versus 1.6 ± 0.5 mmHg, (*P* = 0.027) post intervention in the vitamin D versus placebo group. The estimated mean (±SD) vitamin D intake (2.5 ± 2.6 µg/d) was found to be insufficient compared to the recommended RNI of 10µg/d.

As no significant treatment effect was found with vitamin D₃, a secondary hypothesis was devised to evaluate the association between improvement in 25(OH)D concentration at 4 and 8 weeks with the cardiometabolic markers measured. Thus, correlation analysis with change in 25(OH)D concentration with corresponding changes in the cardiometabolic markers was secondary analysis.

Change in plasma 25(OH)D concentration was inversely associated with change in plasma glucose concentrations (*r* = -0.408, *P* = 0.048), as well as change in HDL-C concentrations (*r* = -0.503, *P* = 0.012) and positively associated with change in PPao

($r = 0.437$, $P = 0.033$) at week 4. Improved 25(OH)D concentration post intervention was inversely associated with change in brachial AIX ($r = 0.446$, $P = 0.029$) and change in plasma glucose concentration ($r = -0.513$, $P = 0.010$). However, the association between change in 25(OH)D and change in HDL-C concentrations, change in PPao, were absent at week 8.

2.4.1. Vitamin D status

In the present study, a daily dose of 5,000IU (125 μ g) vitamin D₃ supplementation increased circulating plasma 25(OH)D concentrations significantly from a baseline mean (\pm SD) concentration of 38.4 ± 15.9 to 64.2 ± 19.5 , ($P < 0.001$) at 4 weeks and 72.8 ± 16.1 nmol/L, ($P < 0.001$) post intervention. At baseline, all recruited participants had plasma 25(OH)D concentration <75 nmol/L, of these, vitamin D deficiency, defined as plasma 25(OH)D concentrations <25 nmol/L (SACN, 2015), was present in 22.2% (12/54) and vitamin D deficiency, defined as plasma 25(OH)D concentrations <30 nmol/L (IOM, 2011) was present in 35.2% (19/54) of the cohort. Plasma 25(OH)D concentration <50 nmol/L, were present in 64.8% (35/54) of the cohort at baseline.

Only 45.8% (11/24) of participants in the vitamin D group achieved plasma 25(OH)D concentrations >75 nmol/L post intervention, despite the high daily dose administered. Evidence from observational studies has suggested a plasma 25(OH)D concentration of at least 75-100nmol/L for extra skeletal benefits (Bischoff-Ferrari, 2008). The United States Endocrine Society's Clinical Practice Guideline recommended a daily dose of at least 6,000 IU (150 μ g) to 10,000 IU (250 μ g) of vitamin D to treat vitamin D deficiency and to maintain serum/plasma 25(OH)D concentrations above 75nmol/L in obese individuals (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, Weaver, et al., 2011). As observed in the present study, a 8 week RCT which administered weekly 50,000IU (1,250 μ g) vitamin D₂ to CVD free overweight and obese individuals with plasma 25(OH)D concentrations below 50nmol/L found a similar increase in plasma 25(OH)D concentrations (Borgi et al., 2016). In this RCT, despite the high weekly 50,000IU (1,250 μ g) vitamin D₂, which amounts to a daily dose of 7,142 IU (178.6 μ g), median (range) plasma 25(OH)D concentration in the vitamin D group was 71.8 (63.5-90.8) nmol/L at 8 weeks compared to the present study mean plasma 25(OH)D

concentration of 72.5 ± 15.7 nmol/L. This finding also supports the hypothesis that vitamin D metabolism is reduced with increasing BMI (Vimaleswaran et al., 2013).

Approximately 54.2% (13/24) of participants in the vitamin D group in the present study did not reach the 75 nmol/L threshold, this could be ascribed to compliance, even though, overall compliance in the vitamin D group was 90%, and those with a percentage compliance $\leq 80\%$ were excluded ($n=1$). Regardless of the high compliance, a major drawback of calculating compliance from returned tablets as was done in the present study, is that it cannot determine whether participants consumed the tablets or discarded them (Chesney et al., 2000; Hill, 2005). Inability to reach the 75 nmol/L threshold could also be ascribed to adipose sequestration, as it has been reported in humans that about 17% of orally-administered vitamin D was stored in adipose tissue (Heaney, Recker, Grote, Horst, & Armas, 2010). Baseline vitamin D status may be another explanation, as it plays an important role in the expected response to vitamin D supplementation, however, only 22.2 % (12/54) participants in the present study had plasma 25(OH)D concentrations <25 nmol/L at baseline, and intestinal absorption of vitamin D may be higher in vitamin D deficient individuals (Apukhovskaia et al., 1990; Heaney, 2012; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011).

The low expression of the 25-hydroxylase CYP2J2 and the 1- α hydroxylase CYP27B1 in obesity may be a potential explanation for the reduced ability of some participants in the vitamin D group to achieve plasma 25(OH)D concentrations above 75 nmol/L. The subcutaneous adipose tissue of obese women has been reported to express lower 25-hydroxylase CYP2J2, as well as the 1- α hydroxylase CYP27B1 compared with the subcutaneous adipose tissue of lean women, indicating that both 25-hydroxylation and 1- α hydroxylation are impaired in obesity (Wamberg, Christiansen, et al., 2013). There is evidence that polymorphisms in the VDBP gene may have an impact on serum 25(OH)D concentrations, as VDBP polymorphisms may reduce the binding capacity of 25(OH)D and thus, reduce circulating plasma 25(OH)D concentrations (Yousefzadeh, Shapses, & Wang, 2014). Although it has been demonstrated that circulating serum VDBP is not affected by adiposity in obese women (Winters, Chennubhatla, Wang, & Miller, 2009), VDBP levels have been reported to be reduced in acute inflammatory conditions (Waldron et al., 2013). This suggests that in obesity, which is characterised by chronic low grade inflammation,

circulating VDBP levels may be reduced (Yousefzadeh et al., 2014), resulting in decreased plasma 25(OH)D concentrations, as VDBP has a greater binding affinity for 25(OH)D compared to 1,25(OH)₂D (Gozdzik et al., 2011; Hart, Furniss, Laurie, & Durham, 2005).

VDR genotype polymorphisms are associated with adiposity (Gu et al., 2009; Ochs-Balcom et al., 2011), and increased production of pro-inflammatory cytokines, as observed with increased adiposity, cause a reduction in VDR expression (Al-Daghri et al., 2014). VDR genotype polymorphism particularly VDR *ff* genotype, have been reported to have decreased response to vitamin D intake (Neyestani et al., 2013). Thus, VDR genotype polymorphism in adiposity may be an additional explanation for the reduced individual response to the high dose orally administered vitamin D intake in the present study.

2.4.2. Effect of vitamin D supplementation on plasma PTH concentrations

Plasma PTH concentrations in this RCT were within the normal range at baseline, and there was no significant change in plasma PTH at 4 and 8 weeks between the groups after vitamin D supplementation. Although it has been reported that serum/plasma PTH concentration is elevated in vitamin D deficiency, serum 25(OH)D concentrations greater than 25nmol/L are not associated with additional change in serum PTH concentrations. Increased serum PTH concentrations have been reported with serum 25(OH)D concentrations below 25 nmol/L (Sayed-Hassan, Abazid, & Alourfi, 2014), and only 22.2% of present study cohort had a plasma 25(OH)D concentration below 25nmol/L. This may explain the normal plasma PTH concentrations, despite low plasma 25(OH)D concentration.

2.4.3. Effect of vitamin D supplementation on haemodynamic measures and plasma sE-selectin concentrations

2.4.3.1 Endothelial function

At baseline, endothelial function, as measured by plasma sE-selectin concentrations (mean \pm SD) were high in the vitamin D group, 57.9 ± 31.1 ng/mL and placebo group 58.7 ± 19.1 ng/mL. This finding supports the evidence that serum sE-selectin concentration is increased in the obese compared to the non-obese (Pontioli, Frige, Paganelli, & Folli, 2009; Zanni, Stanley, Makimura, Chen, & Grinspoon, 2010),

indicating increasing BMI is associated with endothelial dysfunction in adults. No significant effect of vitamin D supplementation was found on endothelial function between treatment groups post intervention. Similar to the present study findings, no significant change in endothelial function, measured by brachial artery ultrasound, was reported following intake of 50,000IU (1,250 µg) vitamin D₂ weekly for 8 weeks in vitamin D deficient CVD-free overweight and obese individuals despite improved vitamin D status (Borgi et al., 2016). Similarly, a 16 week RCT conducted in Singapore in 64 vitamin D deficient T2DM patients did not find significant improvement in endothelial function assessed by reactive hyperaemia index RHI, E-selectin, von-Willebrand factor, hs-CRP and AIx in both treatment groups after administering a daily dose of 4,000IU (100µg) vitamin D₃ (Dalan et al., 2016). From these observations in addition to the present study findings, it could be deduced that low plasma 25(OH)D concentrations may not be the primary cause of endothelial dysfunction as reported by observational studies.

2.4.3.2 Arterial stiffness indices

Mean (\pm SD) PWV (6.5 ± 1.1 m/s), brachial augmentation index ($-48.6 \pm 25.9\%$), central augmentation index ($-12.6 \pm 13.4\%$), RT (153.3 ± 24.4 m/s) and heart rate (62.3 ± 12.3 bpm) were all within the normal ranges at baseline. High daily dose vitamin D₃ supplementation did not improve PWV, AIx, and other indices of arterial stiffness in the present study. These findings may indicate that overall, participants were at low risk of arterial stiffness and cardiometabolic disease. The present study findings are consistent with those of a trial completed in Hong Kong which administered 5,000IU (125µg) oral vitamin D₃ in 100 T2DM patients (Yiu et al., 2013). This study found no difference in endothelial function determined by FMD, PWV or AIx after 12 weeks of intervention (Yiu et al., 2013). However, in this trial, the T2DM patients were taking anti-hypertensive and lipid-lowering drugs which interfere with vitamin D metabolism (Lynch & Price, 2007; Montagnani et al., 1994). Vitamin D is a derivative of cholesterol, and statins lower serum cholesterol concentration and therefore reduce vitamin D synthesis (Istvan & Deisenhofer, 2001). Anti-hypertensive and lipid-lowering drugs may also reduce vitamin D metabolism by competing for CYP3A4 enzyme activity. CYP3A4 is a 25-hydroxylase enzyme that converts inactive to active vitamin D, and is also responsible for the metabolism of antihypertensive drugs as well as statins including

Atorvastatin, Lovastatin and Simvastatin (Bellosta & Corsini, 2012). These drugs are CYP3A4 enzyme inhibitors, and therefore all compete for the same enzyme receptor site, with the more potent drug dominating (Zhou, 2008). This may result in the decreased metabolism of the competing drug which is vitamin D in this case, therefore reducing its efficacy (Zhou, 2008). This could explain the slight increase in serum 25(OH)D concentrations despite the large vitamin D doses administered.

A trial completed in Ireland in 119 vitamin D deficient elderly people did not find significant improvement in PWV and AIx after receiving single dose of either 50,000 IU(1250µg) or 100,000 IU (2,500µg) intramuscular vitamin D₃ for 8 weeks between treatment groups (McGreevy et al., 2015). Arterial stiffness increases with aging, it has been reported to increase between age 50 and 60 years (Cecelja & Chowienczyk, 2012; Reshetnik, Gohlisch, Zidek, Tölle, & van der Giet, 2016), as structural changes in the medial and intimal layer of arteries with increasing age cause the deformation of elastin and collagen fibres and these anatomical changes may not occur in young adults (O’rourke & Hashimoto, 2007; Wang & Lakatta, 2002).

2.4.4. Effect of vitamin D supplementation on blood pressure and RAS

In the present study, mean (\pm SD) SBP (128.7 ± 11.1 mmHg), DBP (77.0 ± 9.7 mmHg), PP (53.6 ± 9.2 mmHg), MAP (95.1 ± 9.1), SBPao (118.5 ± 12.9 mmHg), PPao (41.6 ± 8.8 mmHg), plasma renin concentration (639.8 ± 294 mmHg) and plasma angiotensin II concentrations (32.3 ± 9.6 pg/mL) of overweight and obese cohort were within the normal range. Vitamin D supplementation significantly reduced mean (\pm SD) brachial pulse pressure from baseline (52.3 ± 9.2 vs. 53.7 ± 9.2 mmHg to 49.4 ± 3.9 vs. 53.5 ± 6.4 $P = 0.011$) at 4 weeks and to (50.6 ± 5.8 vs. 55.8 ± 8.7 mmHg, $P = 0.027$) post intervention in the vitamin D compared to placebo group. Although pulse pressure is closely associated with CVD events compared with SBP or DBP (Franklin et al., 2001), it has been reported that central pulse pressure may be a better predictor of CVD risk and not brachial pulse pressure (Cecelja, Jiang, Spector, & Chowienczyk, 2012; Pini et al., 2008). PPao was within the normal range at baseline as mentioned earlier, and remained significantly unchanged post intervention in the present study suggesting the arteries were compliant and distensible.

In the present study, vitamin D supplementation did not significantly reduce SBP, DBP, MAP, SBPao, PPao and RAS in the vitamin D group. Consistent with this finding, a daily dose of 2,000IU (50µg) vitamin D₃ for 6 weeks in 101 stable chronic heart failure patients did not significantly change SBP and DBP. However, contrary to present study findings, this dose significantly reduced median (range) plasma renin concentration from 63 ng/L (38-104) to 55 ng/L (32-93), ($P = 0.020$) in the vitamin D group (Schroten et al., 2013). Similarly, 8 weeks daily supplementation of 2,800IU (70µg) vitamin D₃ in hypertensive patients with low serum 25(OH)D concentration showed no significant effect of intervention on SBP, DBP and plasma renin concentrations (Pilz et al., 2015).

In contrast to the present study findings, weekly 50,000IU (1250µg) vitamin D₃ supplementation for 8 weeks significantly reduced mean (\pm SD) SBP (-6.4 ± 5.3 vs. 0.9 ± 3.7 mmHg, $P < 0.001$), DBP (-2.4 ± 3.7 vs. 1.0 ± 2.7 mmHg, $P = 0.003$) and MAP (-3.7 ± 3.6 vs. 0.9 ± 2.5 mmHg, $P < 0.001$) in the vitamin D compared to placebo group of 42 hypertensive and vitamin D deficient Iranian out-patients (Mozaffari-Khosravi, Loloei, Mirjalili, & Barzegar, 2015). However, this study did not find a significant impact of vitamin D supplementation on pulse pressure (Mozaffari-Khosravi et al., 2015). The use of a non-hypertensive cohort at baseline may be the possible reason the present study did not find significant changes in SBP, DBP and MAP compared to the aforementioned study. The present study used participants with normal baseline SBP, DBP and MAP, this suggest that vitamin D may provide significant reductions in BP only in hypertensive individuals. In agreement with this hypothesis, a meta-analysis of 11 RCTS found non-significant reductions in SBP and significant reductions in DBP in the vitamin D group, in 8/11 RCTs in which mean baseline BP was >140 mmHg (Witham, Nadir, & Struthers, 2009). Findings from an observational study have also demonstrated that low serum 25(OH)D concentrations were a much stronger predictor of future CVD in people with hypertension at baseline compared to normotensive people (Wang, Pencina, et al., 2008). It has been demonstrated *in vivo*, that arterial stiffness is increased by a significant rise in blood pressure (Stewart, Millasseau, Kearney, Ritter, & Chowienczyk, 2003), and that arterial stiffness is greater in hypertensive individuals compared to age-matched normotensive controls (Stewart, Jiang, Millasseau, Ritter, & Chowienczyk, 2006). From these observations, the lack of an effect of vitamin D

supplementation in the present study, could be attributed to the use of young non-hypertensive overweight and obese study cohorts, consequently this study cohort would have had fewer anatomical changes to their arterial tree as majority of cohort were below 50 years.

2.4.5. Effect of vitamin D supplementation on oxidative stress and inflammation

The present study observation of high baseline plasma mean (\pm SD) hs-CRP concentrations (2.9 ± 1.9 mg/L) is consistent with findings that increasing BMI, characterised with excess adipose tissue is associated with abnormal cytokine production and increased secretion of acute phase reactants as well as the upregulation of the inflammatory signalling pathway (Berg & Scherer, 2005; Xu et al., 2003). Consistent with this observation, overweight and obese women have been reported to have increased serum TNF- α (inflammatory marker) concentrations compared to lean women (Olszanecka-Glinianowicz, Zahorska-Markiewicz, Janowska, & Zurakowski, 2004). Additionally, subcutaneous and visceral adipocyte cell volume demonstrated a higher positive association with serum TNF- α concentration in overweight/obese adults compared to lean controls (Winkler et al., 2003). In the present study cohort, mean (\pm SD) plasma concentration of 8-isoprostanes 11.2 ± 8.7 mg/L were within the normal range and this observation was unexpected, as increased secretion of fatty acids by adipocytes have been reported to induce the production of ROS by upregulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and by downregulating the expression of anti-oxidative enzymes thereby increasing oxidative stress (Goncharov et al., 2015). The present study demonstrated that vitamin D supplementation did not significantly reduce plasma hsCRP concentration and significantly change plasma 8-isoprostanes concentrations at 4 and 8 weeks. These results are in line with a 12 week trial which did not also observe a significant effect of daily 5,000IU vitamin D₃ supplementation on hs-CRP and 8-isoprostanes in 100 T2DM patients (Yiu et al., 2013). This finding may suggest vitamin D supplementation does not reduce or prevent inflammation in overweight and obese people. The normal baseline plasma 8-isoprostanes concentration may also explain why arterial stiffness indices were within the normal ranges, as increased oxidative stress is implicated in the irreversible cross-linking of collagen and elastin fibres by advanced glycation end products (Konova et al., 2004).

2.4.6. Effect of vitamin D supplementation on anthropometric measurements

In the present study, despite improvements in vitamin D status, no significant reductions were observed in BMI, body weight and waist circumference between treatment groups. This observation is consistent with the results of a US RCT which demonstrated that a daily dose of either 1,000IU (25µg), 2,000IU(50µg) or 4,000IU (100µg) for 3 months did not significantly reduce BMI in 328 overweight African-Americans (Chandler et al., 2015). Similarly, a meta-analysis of 12 RCTs performed in Australia showed no significant effect of vitamin D therapy on body weight, BMI, or other adiposity measures in the absence of calorie restriction (Pathak, Soares, Calton, Zhao, & Hallett, 2014). Contrary to these findings, an Iranian RCT in 77 healthy overweight and obese women showed 7% reductions in body fat mass after study cohort received 1000 IU (25 µg) vitamin D₃ for 12 weeks, however, the vitamin D intervention did not significantly affect body weight and waist circumference (Salehpour et al., 2012).

In the present study, obese participants (n = 18) at baseline had plasma mean (\pm SD) 25(OH)D concentrations of 37.9 ± 17.2 nmol/L, whilst those in the overweight category (n = 36) had slightly higher mean (\pm SD) plasma 25(OH)D concentrations of 41.7 ± 20.2 nmol/L. Post intervention, 4 out of the 18 obese cohort, achieved a plasma 25(OH)D concentration ≥ 75 nmol/L. The present study findings are consistent with a bi-directional Mendelian randomisation meta-analysis study using genetic markers. This meta-analysis demonstrated that a higher BMI would result in a low vitamin D status, but a lower vitamin D status was unlikely to increase BMI in 21 cohorts of over 42,024 participants (Vimaleswaran et al., 2013). The lack of an effect of vitamin D supplementation on BMI, body weight and waist circumference may also be attributed to the method used in assessing body fat, the present study used BMI in calculating body fat but did not measure body fat distribution. Although BMI measurement is inexpensive, quick and easy tool to evaluate obesity trends in the general population, it can overestimate or underestimate body fat levels, due to inability to distinguish between muscle and lean mass for fat distribution (Ashwell, 2011; Bhurosy & Jeewon, 2013).

2.4.7. Effect of vitamin D supplementation on lipid profile

Serum mean (\pm SD) of lipid profile biomarkers reported in the recent NDNS for the corresponding sex and age groups are 4.9 ± 1.5 , 1.3 ± 0.4 and 3.0 ± 0.9 mmol/L for TC, HDL-C and LDL-C respectively. Compared with the general UK male adult population, the results of lipid profile markers indicate that participants were at high risk of cardiometabolic disease as shown by elevated mean (\pm SD) TC (6.5 ± 1.5 mmol/L), LDL-C (5.2 ± 1.5 mmol/L), non-HDL (6.3 ± 1.8 mmol/L), reduced HDL-C (0.6 ± 0.1 mmol/L), and borderline serum TAG (1.7 ± 0.4 mmol/L). Lipid profile markers have been shown to be unfavourable in the overweight and obese, as serum TC, TAG and LDL-C concentrations were found to be higher in the obese compared to normal weight individuals, whilst serum HDL-C concentration was lower in the obese compared to non-obese people (Klop, Elte, & Cabezas, 2013; Szczypińska, Widomska, Jaraszkiewicz, Knera, & Muc, 2003). Plasma HDL-C and apolipoprotein A-1 (the primary protein fraction of HDL-C) concentrations have been reported to both significantly decrease with increasing BMI. Increase in BMI is strongly associated with reduced serum HDL-C concentration compared to high LDL-cholesterol concentrations (Klop et al., 2013). The potential mechanisms responsible for reduced serum HDL-C concentration in obese states include: Obesity alters the cardioprotective HDL-C subfractions such as HDL₂ apolipoprotein A-I, and pre- β_1 , and reduced serum HDL-C concentrations in obesity have been attributed to both an enhanced uptake of HDL₂ by adipocytes as well as the increased catabolism of apolipoprotein A-I subfraction (Rashid & Genest, 2007).

There was no effect of vitamin D supplementation on lipid profile markers in the vitamin D group. Consistent with the present study, a 16 weeks Iranian RCT in 71 males and females with metabolic syndrome showed that 50,000IU (1250 μ g) vitamin D₃ did not significantly improve serum mean (\pm SD) TC, HDL-C, and LDL-C concentration (Salekzamani et al., 2016). Similarly, a UK trial in 50 chronic fatigue syndrome patients who received 100,000IU of vitamin D₃ every 2 months for 6 months did not demonstrate any effect of treatment on lipid profile (Witham et al., 2015). Additionally, a 8-week trial in 60 centrally obese people showed that 1,200IU (30 μ g)/day vitamin D₃ did not increase serum 25(OH)D concentrations to a desirable concentration of ≥ 75 nmol/L in 61% of participants and there was also no significant changes in lipid profile biomarkers (Maki et al., 2011).

2.4.8. Effect of vitamin D supplementation on metabolic profile markers

In the present study, the cohort was non-diabetic and had a normal mean (\pm SD) baseline HOMA-IR (1.6 ± 1.3), fasting plasma mean (\pm SD) glucose (5.2 ± 1.5 mmol/L) and mean (\pm SD) insulin concentrations (46.5 ± 27.7 pmol/L). There was no effect of vitamin D supplementation on fasting plasma glucose and plasma insulin, HOMA-IR in the vitamin D group. In agreement with the present study findings, supplementation with a weekly dose of 88,865 IU ($2,222\mu$ g) for 12 months had no effect on fasting plasma glucose, insulin secretion and insulin sensitivity in overweight people with prediabetes and hypovitaminosis D ($25(\text{OH})\text{D} < 75$ nmol/L) (Davidson, Duran, Lee, & Friedman, 2013). Similarly, a meta-analysis of 12 RCTs in overweight and obese people demonstrated that vitamin D supplementation with varying doses, duration and baseline serum $25(\text{OH})\text{D}$ concentration had no significant effect on serum/plasma fasting glucose, insulin and HOMA-IR index (Jamka, Woźniewicz, Jeszka, et al., 2015).

The present study may not have observed significant reductions with vitamin D supplementation in the glucose homeostasis parameters measured, as the study cohort were non-diabetic and had normal baseline HOMA-IR, fasting plasma glucose and insulin concentrations. It may be possible that vitamin D supplementation may be effective in insulin resistant individuals, as 6 months administration of 4,000IU (100μ g) vitamin D_3 significantly reduced fasting insulin concentration and significantly improved insulin sensitivity and resistance in insulin resistant and vitamin D deficient ($25(\text{OH})\text{D} < 50$ nmol/L) South Asian women (von Hurst et al., 2010). This RCT also observed that insulin resistance was most reduced at a serum $25(\text{OH})\text{D}$ concentration of 80-119nmol/L, suggesting a dose response relationship between vitamin D status and insulin resistance (von Hurst et al., 2010).

2.4.9. Associations between plasma $25(\text{OH})\text{D}$ concentration and the cardiometabolic markers measured

In the present study, baseline plasma $25(\text{OH})\text{D}$ concentration was inversely associated with fasting plasma insulin concentration ($r = -0.307$, $P = 0.024$) and HOMA-IR ($r = -0.307$, $P = 0.024$), indicating that decreasing $25(\text{OH})\text{D}$ concentration is associated with markers of impaired glucose metabolism (Dalgård, Petersen, Weihe, & Grandjean, 2011; Forouhi, Luan, Cooper, Boucher, & Wareham, 2008). As

observed in the present study, serum 25(OH)D concentration below 50nmol/L was inversely associated with HOMA-IR ($r = -0.350$, $P < 0.001$) and with fasting plasma insulin concentration ($r = -0.350$, $P < 0.001$) in a cross-section of 50 obese and 36 normal weight adult non-hypertensive and non-diabetic patients (Stokić et al., 2015). Additionally, a cross-sectional study in overweight and obese elderly Faroese residents also reported inverse associations between serum 25(OH)D concentration with fasting plasma insulin ($r_s = -0.10$; $P = 0.01$) and HOMA-IR ($r_s = -0.10$; $P = 0.01$) (Dalgård et al., 2011).

In the present study, change in plasma 25(OH)D concentration at week 4 was inversely associated with change in plasma glucose concentrations ($r = -0.408$, $P = 0.048$), suggesting increasing 25(OH)D concentration is associated with improved glucose metabolism. A cross-sectional study in 80 post menarchal obese adolescents showed that serum 25(OH)D concentrations was inversely associated with fasting glucose ($r = -0.28$, $P = 0.02$) (Ashraf, Alvarez, Gower, Saenz, & McCormick, 2011). A cross-sectional study in CVD and systemic disease free severe obese adults also found inverse associations between median plasma 25(OH)D concentrations and fasting glucose -0.0022 (-0.0091 to -0.0016), ($P = 0.033$) (Bellia et al., 2013). The mechanism by which improved vitamin D status influences metabolic indices of glucose directly involves the stimulation of β -cell function which in turn improves insulin secretion, insulin sensitivity and insulin-mediated uptake of glucose in adipose and muscle tissue (Alvarez & Ashraf, 2009; Bajaj et al., 2014; Mitri et al., 2011).

Change in plasma 25(OH)D concentration at week 4 was also inversely associated with change in plasma HDL-C concentrations ($r = -0.503$, $P = 0.012$). This observation is contrary to those of a large cross-sectional study of 4342 non-pregnant women who were categorised into abdominally normal, overweight and obese. This study found lower serum 25(OH)D concentrations was significantly associated with lower HDL-C, suggesting higher serum 25(OH)D concentration would in turn be associated with higher serum HDL-C concentrations (Vogt, Baumert, Peters, Thorand, & Scragg, 2016). Additionally, in 1534 hyperlipidaemic patients it was demonstrated that serum 25(OH)D concentrations was positively associated with HDL-C ($r = -0.190$, $P < 0.0001$) (Glueck et al., 2016). These observations indicate that a higher serum 25(OH)D concentrations are associated with lower CVD risk

(Cheng et al., 2010). Additionally, change in plasma 25(OH)D concentration at week 4 was positively associated with change in aortic central pulse pressure ($r = 0.437$, $P = 0.033$). Contrary to the observations in the present study, a significant inverse association between low serum 25(OH)D and central pulse pressure was demonstrated in a cross-section of south African women ($r = -0.157$; $P = 0.025$) (Kruger et al., 2013). Contrary to these findings, results from the third US National Health and Nutrition Examination Survey (NHANES III) did not find an inverse association between serum 25(OH)D and central pulse pressure (Scragg, Sowers, & Bell, 2007).

Change in plasma 25(OH)D concentration at 8 weeks was inversely associated with change in brachial augmentation index ($r = -0.446$, $P = 0.029$). As observed in the present study, a study in which brachial and central augmentation index standardised to a heart rate of 75bpm was found to be positively associated with bioavailable 25(OH)D ($\rho=0.4$, $P=0.004$) and free 25(OH)D concentrations ($\rho=0.4$, $P=0.009$) in a cohort of 47 post-menarchal, adolescent girls (Ashraf et al., 2014). Taken together, these observations suggest that arterial stiffness increases or decreases with decreasing or increasing plasma 25(OH)D concentrations. A cross-sectional analysis in 190 obese youth with and without T2DM observed an inverse association between serum 25(OH)D concentration and AIx in lean and T2DM (both $r = -0.24$, $P < 0.01$), and increased serum 25(OH)D concentration of 7.5nmol/L in the obese youths with T2DM was associated with a 1% reduction in AIx (Jha et al., 2015).

Potential mechanisms by which improved vitamin D exerts beneficial effects on indices of arterial stiffness involves stimulating the production of endothelial nitric oxide (Kassi et al., 2013; Norman & Powell, 2005) and by downregulating the renin-angiotensin system (Li, 2003). Improved vitamin D status exerts beneficial effects on LDL-C by modulating inflammatory processes and lipid metabolism (Gonzalez & Moschetta, 2014). Vitamin D may also exert beneficial effects by directly regulating the VSMC production (Mayer et al., 2012), as well as by obstructing the adverse effect of advanced glycation end-products on vascular aging (Talmor et al., 2008).

Although, this RCT was not designed to investigate the dose response association between baseline plasma 25(OH)D concentrations and the prevention of CVD, it is important that the optimal serum/plasma 25(OH)D concentrations required to prevent

CVD risk be determined, as a threshold effect which varies with different baseline serum/plasma 25(OH)D concentrations has been reported (Heaney, 2012; Mozos & Marginean, 2015). A meta-analysis consisting of 18 observational studies has shown that metabolic risk decreases with increasing serum/plasma 25(OH)D concentration, this study compared the association between different serum 25(OH)D concentration with metabolic syndrome risk. Metabolic syndrome risk was reported to be 0.89 (95% CI=0.85–0.93) for a serum/plasma 25(OH)D concentration of 30 nmol/L, 0.84 (95% CI= 0.74–0.96) of 40 nmol/L, 0.80, 95% CI = 0.73–0.87 for 60 nmol/L 0.70 (95%CI= 0.56–0.87) for 70 nmol/L, 0.67 (95% CI = 0.52–0.80) for 100 nmol/L, and 0.60 (95% CI= 0.44–0.82) for 145 nmol/L (Ju, Jeong, & Kim, 2013). Additionally, a meta-analysis of 11 observational studies reported that a serum/plasma 25(OH)D concentration of 75-87.5 nmol/L reduces the risk of CVD mortality (Zittermann et al., 2012). Furthermore, a meta-analysis of 19 observational studies demonstrated that the increased CVD risk with low serum 25(OH)D was generally linear over a serum 25(OH)D threshold of 20 to 60 nmol/L (Wang, Song, et al., 2012).

2.4.10 Dietary intake

Baseline dietary nutrient analysis of mean energy, carbohydrate, protein, fat and vitamin D intake based on reported consumption from the 3 day food diary show that the mean daily energy intake for males in the cohort aged 19-64 years (n = 42) was mean \pm SD: 8.23 ± 2.62 MJ/day. These values are comparable to that reported for the corresponding sex and age group in the recent NDNS, which is 8.86 ± 2.36 MJ/day (Public Health England, 2016). The estimated mean dietary carbohydrate intake of (219.4 ± 67.5 g/day) was below the DRV and the UK (257 ± 76 g/day) estimated average requirement for carbohydrate for males aged 19-64. From the results, it could be deduced that the participants did not obtain their average daily total energy intake from carbohydrate. Additionally, the reduced estimated mean carbohydrate intake by cohort in the present study could be attributed to under reporting, the study population were overweight or obese males and hence they may under report to prevent judgement of eating too much (Johansson, Solvoll, Bjørneboe, & Drevon, 1998; Scagliusi, Polacow, Artioli, Benatti, & Lancha, 2003). The mean (\pm SD) dietary protein intake 97.1 ± 46.9 g/day was above those of the general UK adult male population of 84.6 ± 17.6 g/day and above the DRV (55.5g/day) for males. This result suggests majority of participants obtained their average daily total energy

intake from dietary protein. The mean (\pm SD) dietary fat intake (76.2 ± 23.5 g/day) was within the range of the general UK adult male population (76.3 ± 5.5 g/day) for corresponding age, suggesting, that majority of participants also obtained their energy requirements from fat. The estimated mean (\pm SD) daily intake of vitamin D in the present study cohort (2.5 ± 2.6 μ g/d) was inadequate compared with the RNI value of 10 μ g/d (SACN, 2016), suggesting the cohort obtained very little vitamin D from their diet. However, a 3-day food diary only estimated mean dietary vitamin D intake for 3 days of the week, hence, the determination of dietary contribution of vitamin D may be underestimated. Under-reporting whilst partaking in a study is common with the use of a 3 day diary in the overweight and obese, as they are more likely to alter their eating habits and only record socially acceptable food and drinks rather than those actually consumed (Ortega, Perez-Rodrigo, & Lopez-Sobaler, 2015).

2.4.11. Study strengths

To the best of my knowledge, this is the first RCT investigating in non-hypertensive and non-diabetic overweight and obese adult males the effect of daily 5,000IU (125 μ g) vitamin D₃ on a broad range of cardiometabolic risk markers. The randomised double- blind, placebo-controlled design is another strength of the present study, as it allows causal inferences to be drawn, therefore, it is the strongest and reliable evidence of a treatment effectiveness (Levin, 2007). The present study collected information about dietary intake using a 3-day food diary, thus, the possible influence of a differential vitamin D intake was excluded. Although not a gold standard method of measuring total 25(OH)D, the present study measured 95% of participants' plasma 25(OH)D concentration using VIDAS[®] Total 25(OH)D assay kit, which accurately determines both D₂ and D₃ in human serum or plasma using the Enzyme Linked Fluorescent Assay (ELFA) technique. VIDAS[®] Total 25(OH)D kit is very well correlated to the Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) reference method, a high positive association was recorded ($Y=1.00x+ 041$, $R = 0.86$) when 343 samples were tested using VIDAS[®] Total 25(OH)D and LC-MS/MS (the gold standard method). VIDAS[®] Total 25(OH)D assay kit also provides the same high degree of precision as the LC-MS/MS, it is easy to perform in any lab and gives rapid results within 40 minutes using the Mini-vidas, a multi parametric immunoanalyser. The laboratory used for

the present study is accredited by DEQAS, an accuracy-based program for improving the comparability of laboratory measurements for 25(OH)D assays, which have values assigned by the NIST reference measurement procedure. A certificate has been awarded to our laboratory, as it met the performance targets.

The measurement of PWVao, AIx and other arterial stiffness indices using the Arteriograph (cuff based oscillometric device) is a study strength. The use of Arteriograph, is a simpler and less technical method compared to Complior and sphygmoCor (applanation tonometry devices), the most often used devices which are more complex, require sophisticated equipment and trained personnel that limits their use in clinical studies (Baulmann, Schillings, Rickert, Uen, Düsing, et al., 2008), as they require the device sensors to be accurately placed on the carotid and femoral arteries (Jatoi, Mahmud, Bennett, & Feely, 2009). The use of the Arteriograph or oscillometry has been proposed as a new gold standard for PWV measurement, as technique is simple to use, reliable and accurate and would be more suitable in clinical practice and in clinical studies (Davies et al., 2012; Reshetnik et al., 2016).

An Arteriograph validation study which measured PWV and AIX 5 times in 51 patients and repeated measurements after 1 week in 35 patients by comparing it to clinically validated broadly accepted systems (SphygmoCor and Complior) showed that the correlations of PWV measured by Arteriograph with the values obtained using the SphygmoCor ($r = 0.67$, $P < 0.001$) and the Complior ($r = 0.69$, $P < 0.001$) were highly significant (Baulmann, Schillings, Rickert, Uen, Dusing, et al., 2008). AIX values obtained between the Arteriograph and SphygmoCor were very closely related ($r = 0.92$, $P < 0.001$) (Baulmann, Schillings, Rickert, Uen, Dusing, et al., 2008).

The measurement of the selected cardiometabolic markers at multiple time points is another advantage, as it controls for factors that cause variability between subjects and can track an effect overtime (Hoyland, Dye, & Lawton, 2009), as well as eliminate error in measuring (Bhutta et al., 2013).

Sample size calculation for the present study was based on change in FMD, an endothelial function/CVD marker, whereas most RCTs investigating the effect of vitamin D supplementation on cardiometabolic risk were originally powered based on muscle function and skeletal outcomes and not CVD outcomes. The present study

used population of overweight and obese adult males with a high prevalence of both vitamin D deficiency and increased CVD and metabolic risk, thus, the beneficial effect of vitamin D treatment would be significant in this cohort and the likelihood of reverse causation would be decreased. The daily high dose administered is additional study strength, as most RCTs in the overweight and obese have administered lower daily doses or single large doses. When a post hoc was performed using mean (\pm SD) of sE-selectin concentrations post intervention for vitamin D; 47.2 ± 32.5 ng/mL and placebo group; 41.7 ± 22.0 ng/mL, given the present study sample size, the achieved level of power was 0.20. This indicates that sample size was too small to gain a significant value due to PhD time and budget restraints. To achieve a 0.80 power a sample size of 40 in each intervention arm would be required and this would be an effective future study.

2.4.12 Study limitations

There are several possible reasons for the lack of beneficial effects of vitamin D supplementation on cardiometabolic risk markers in this trial. Firstly, vitamin D supplementation has been reported to be effective in people with low baseline serum 25(OH)D concentration, and only 22.2% (11/54) and 33.3 % (18/54) of present study participants had a plasma 25(OH)D concentration < 25 nmol/L and < 30 nmol/L respectively, independent of treatment group. Thus, it was not possible to test this hypothesis from the present study. A larger dose of vitamin D and a longer duration of supplementation may be required, as plasma 25(OH)D concentrations > 75 nmol/L are required for optimal extra-skeletal health (Bischoff-Ferrari, 2008). In the present study, BMI was measured, but percentage of body fat distribution was not measured because as at the time of data collection, equipment for measuring body fat distribution was not available. Most of the study participants were < 50 years and physically active, thus they may have less established structural changes in their arterial tree as demonstrated by the vascular markers. Since cardiometabolic risk increases with age, and only a small number of participants over 50 years were recruited, the ability to detect significant differences in this age group was limited.

Furthermore, evidence suggests that altered vitamin D homeostasis may contribute to increased CVD risk with increasing BMI, this association has been observed with serum 25(OH)D concentrations < 43 nmol/L (Snijder et al., 2005), and only 53.7% of

study participants had plasma 25(OH)D concentrations $<43\text{nmol/L}$ in both groups at baseline. Finally, the present study did not investigate the role of VDR genotype polymorphism in determining plasma 25(OH)D concentrations in adiposity (Ochs-Balcom et al., 2011), as it has been reported to affect individual response to vitamin D intake in obese individuals (Neyestani et al., 2013).

2.4.13. Future perspectives

It is highly recommended that a threshold value for 25(OH)D concentration be determined to prevent atherosclerosis and reduce cardiometabolic risk in overweight and obese individuals. No significant improvement in arterial stiffness, as assessed by PWV and AIx and other indices using the Arteriograph was observed in this RCT, thus, further research using FMD and other devices of measuring arterial stiffness are required in this cohort. RCT studies involving a large number of overweight and obese people are required to generalise the results of this study. RCT studies using a similar population with vitamin D deficiency and CVD disease may show significant impact of vitamin D supplementation. It would be important to measure circulating VDBP concentrations in the overweight and obese males, as it has been reported to be reduced in acute inflammatory conditions. Few RCTs in overweight and obese people have focussed on the effect of VDR genotype polymorphisms on circulating plasma 25(OH)D concentration. Measuring VDR genotype polymorphisms may be significant in understanding the variations in individual response to oral vitamin D supplementation in the overweight and obese.

2.4.14 Conclusion

Overall, the findings from this RCT demonstrate that a daily dose of 5,000IU (125 μg) vitamin D₃ for 8 weeks increased plasma 25(OH)D concentrations to $\geq 75\text{nmol/L}$ in 45.8% of the study cohort, but did not significantly affect the cardiometabolic risk markers measured except for brachial pulse pressure. However, there is suggestion that baseline plasma 25(OH)D concentration influences the effect of vitamin D repletion on cardiometabolic markers. Improved vitamin D status was inversely associated with change in plasma glucose concentrations ($r = -0.408$, $P = 0.048$), and with change in HDL-C concentrations ($r = -0.503$, $P = 0.012$) and positively associated with change in aortic central pulse pressure ($r = 0.437$, $P = 0.033$) at week 4. Improved vitamin D status post intervention was inversely

associated with change in brachial augmentation index ($r = -0.446$, $P = 0.029$). However, the association between change in 25(OH)D and change in HDL-C concentrations, change in PPao, were absent at week 8.

The absence of a significant treatment effect on the selected cardiometabolic markers suggests that vitamin D supplementation cannot be effectively used to prevent cardiometabolic disease risk in healthy overweight/obese males. As a result, the present study does not support the hypothesis that high daily dose vitamin D supplementation reduces or prevents cardiometabolic disease risk in a cohort of non-hypertensive and non-diabetic overweight and obese adult males. The estimated data for mean dietary intake of vitamin D in the cohort was inadequate compared to the recommended RNI for vitamin D in the general UK population. As a result of the variations in vitamin D metabolism in overweight and obese people compared to normal weight people, and a reported low mean dietary vitamin D intake, it is important that a specific higher daily dose of vitamin D be recommended for the overweight and obese. Finally, the association between serum/plasma 25(OH)D concentrations and various health outcomes as reported among systematic reviews and meta-analyses from observational studies and RCTs provides little evidence of an association between vitamin D and various obesity-related outcomes including cardiometabolic disease (Theodoratou et al., 2014). A possible explanation for this difference may be that obesity-related clinical disease leads to decreased vitamin D concentrations, producing insufficiency, rather than causing ill health (Theodoratou et al., 2014).

**3.0 VITAMIN D STATUS AND
CARDIOVASCULAR DISEASE RISK IN A
COHORT OF CALCIUM OXALATE
KIDNEY STONE DISEASE PATIENTS.**

3.0 VITAMIN D STATUS AND CARDIOVASCULAR DISEASE RISK IN A COHORT OF CALCIUM OXALATE KIDNEY STONE DISEASE PATIENTS.

3.1 INTRODUCTION

Kidney stones are hard crystalline mineral deposit that develops within the kidney or urinary tract. They are formed when urine becomes supersaturated with the salt and minerals secreted by the kidneys due to an imbalance between the precipitation and solubility of these salts (Han, Segal, Seifter, & Dwyer, 2015; Reid, Jackson, Duer, & Rodgers, 2011). Kidney stone disease are highly prevalent systemic disorder with a major health and economic burden (Keddis & Rule, 2013). There is a gradual increase in kidney stone incidence and prevalence globally (Romero, Akpinar, & Assimos, 2010), and approximately 1-5% of populations in Asia, 5-9% in Europe, 10-15% in the USA and 20-25% of populations in the Middle East are affected, with the smallest prevalence been recorded in Japan and Greenland (Najeeb et al., 2013). Over the last 10 years there has been a 63% growth in the prevalence, with 720,000 people estimated to be living with kidney stones in the UK (Romero et al., 2010; Turney, Reynard, Noble, & Keoghane, 2012).

Kidney stone incidence is influenced by age, sex, race and ethnicity, formerly, Caucasian males between the ages of 20 and 60 had a higher incidence compared to young African American females (Dawson & Tomson, 2012; Lieske et al., 2006; Romero et al., 2010). However, recently the gap has closed, as the incidence in females is almost equivalent to that of males, around 3 in 20 males and 1 in 20 females develop kidney stones in the UK (Turney et al., 2014). Without medical treatment there is a 50% risk of kidney stones reoccurring within 5-10 years and 75% likelihood within 20 years, however, in most cases this depends on the disease causing the stone formation (Dawson & Tomson, 2012). The incidence of kidney stones depends on risk factors including dehydration, urinary tract infections, low urine volume and calcium intake, geographical, genetic and dietary factors amongst others (Dawson & Tomson, 2012; Fakheri & Goldfarb, 2011; Wang, Chen, Song, Caballero, & Cheskin, 2008). The incidence of kidney stones is expected to rise following the global increase in people being overweight and obese and the improvement in socioeconomic status of the population (Najeeb et al., 2013).

Kidney stone disease is a risk factor for chronic renal disease, as kidney stones can result in the development of chronic tubule-interstitial nephritis, estimated to account for 15-30% of end stage chronic insufficiency known to give rise to end stage renal disease (Dardamanis, 2013). Polymorphisms and mutations of CYP24A1 genes have been implicated as a further factor responsible for the high risk of kidney stones (Dinour et al., 2013), because variants of the CYP24A1 gene have been identified in a small number of calcium kidney stones formers (Sayers, Hynes, Rice, Hogg, & Sayer, 2013).

3.1.1 PATHOMECHANISMS OF KIDNEY STONE FORMATION

Urinary supersaturation is the primary stimulus for crystallisation (Aggarwal, Narula, Kakkar, & Tandon, 2013). A solution is considered saturated when it has reached a particular concentration beyond which no further dissolution is possible (Aggarwal et al., 2013). At this degree of saturation if more salts are added to the saturated solution, it will precipitate and form crystals as long as the temperature and pH remain constant and if inhibitors of crystallisation do not act at this stage kidney stones will be formed (Aggarwal et al., 2013). The pathomechanisms of stone formation include crystal nucleation, aggregation and crystal growth, although a mechanism for the high degree of supersaturation of stones differs depending on the stone type (Dardamanis, 2013).

3.1.1.1 Crystal nucleation

Nucleation is the initial step in the change from a liquid to a solid phase in a supersaturated solution (Dardamanis, 2013). The nucleus is produced from microcrystals formed by the supersaturation of stone forming salts such as calcium, oxalate and cysteine in the urine (Sakhaee, 2009). The nucleus usually forms on existing surfaces in urine, a process known as heterogeneous nucleation, although it is also capable of forming on epithelial cells, urinary casts, red blood cells, amongst others in urine (Dardamanis, 2013). The degree of saturation required for heterogeneous nucleation is much less than for homogenous nucleation (Smith, 1990). Once a nucleus is formed and anchored, crystallisation possibly occurs at lower chemical pressures than that required for the formation of the initial nucleus (Fasano & Khan, 2001). Renal tubular cell injury is capable of inducing the crystallisation of CaOx crystals by providing substances for their heterogeneous

nucleation (Aggarwal et al., 2013). Following renal tubular cell injury, *in vitro* cell degradation produces several membrane vesicles, reported to be good nucleators of calcium crystals. (Fasano & Khan, 2001).

3.1.1.2 Aggregation

Aggregation, a critical step, is the process by which microcrystals (nucleus) in solution increase in size to form a large mass made up of proteins, lipids, polysaccharides and other cell derived materials by interacting with each other (Aggarwal et al., 2013; Dorian, Rez, & Drach, 1996). To have the capacity to cause occlusion in the distal nephron, the formed mass must be large. Aggregation also involves the secondary nucleation of new crystals on the surface of already formed crystals (Gower, Amos, & Khan, 2010; Sandersius & Rez, 2007). For aggregation to occur supersaturation of urine, lack of inhibitors of nucleosis and the type of organic substrate and epitaxis are required for crystals to adhere to the epithelial surface of crystals of a different chemical structure, for example the adherence of calcium oxalate crystals onto struvite crystals (Dardamanis, 2013).

3.1.1.3 Crystal growth and crystal cell interaction

Crystal growth is an essential phase for kidney stone formation. It is the process by which new crystal components are added to an existing crystal. It occurs after a crystal must have attained a pathophysiological critical size by aggregation and with the relative supersaturation still remaining above one (Hu, Hu, Wang, Lu, & Qin, 2011). Crystal cell interaction occurs when microcrystals formed from urine supersaturation attach and adhere to renal tubular epithelial cells. Crystal-cell interactions is also an important early process in the development of kidney stones (Khan et al., 1999).

3.1.2 Promoters of kidney stone formation

Promoters bring about stone formation, as they increase the rate of crystallisation or growth of the constituents of kidney stones (Khan & Kok, 2004). Urinary saturation can be raised by increasing the concentration of the reactants. Urine may contain substances that are capable of lowering the formation product, however, the formation product may also be lowered by the absence of endogenous inhibitors, or obstruction to their effects by defects in their structure or other interfering substances

(Farell, Huang, Kim, Horstkorte, & Lieske, 2004). Extremely high urinary pH, low urine volume and urate/uric acid may promote the formation of heterogeneous nucleating substances (Farell et al., 2004; Moe, Abate, & Sakhaee, 2002).

A highly acidic urine causes the precipitation of uric acid crystals which may lead to uric acid stone formation and may also enhance calcium oxalate crystallisation (Moe et al., 2002). Highly alkaline urine may also promote secondary nucleation of calcium oxalate by precipitation of calcium phosphate (Ratkalkar & Kleinman, 2011). Reduced urinary volume resulting from either inadequate intake or excessive loss of liquid through the intestinal tract or sweating would intensify the saturation of all solutes, in turn increasing the risk of the various type of kidney stones formation (Moe, 2006). In addition to low urine volume, uric acid or monosodium urate enhances the attachment of calcium oxalate to cells whilst promoting heterogeneous nucleation (Farell et al., 2004).

3.1.3 Inhibitors of kidney stone formation

The various types of kidney stone inhibitors in urine include osteopontin (OPN) nephrocalcin (NC), Tamm-Horsfall protein (THP) and urinary prothrombin fragment 1 (UPTF1) (Ratkalkar & Kleinman, 2011). These inhibitors have been proposed to protect the body against kidney stones development ; abnormalities of these inhibitors may lead to stone formation, growth and also calcification (Dardamanis, 2013).

3.1.3.1 Tamm- Horsefall protein

THP is a major component of kidney stones regardless of the stone type (van Rooijen, Voskamp, Kamerling, & Vliegenthart, 1999), and is capable of inhibiting the aggregation of crystals, thus, making them unable to form a mass big enough to cause occlusion (Viswanathan et al., 2011). However, THP is also capable of acting as a promoter of crystal deposition, depending on the experimental condition (Grover, Ryall, & Marshall, 1990; Viswanathan et al., 2011). One isoelectric focusing study which chemically desialylated (removal of sialic acid residues) THP isolated from a healthy individual showed that desialylated THP promoted calcium oxalate monohydrate aggregation, whilst normal THP inhibited aggregation

(Viswanathan et al., 2011). It has been hypothesised that the THP of stone formers is structurally different from that of the healthy populations (Hess, 1991).

3.1.3.2 Nephrocalcin

NC is a principal inhibitor of urinary calcium oxalate (CaOx) crystallisation, as its activity is responsible for approximately 90% of urine's total inhibitory effect on calcium oxalate crystallisation (Aggarwal et al., 2013). NCs potent ability to inhibit CaOx crystallisation is mediated through its Gla component (Edson et al., 2013). It has been suggested that the absence of Gla component in nephrocalcin isolated from CaOx kidney stones could be the reason for the formation of the kidney stones (Jonassen, Kohjimoto, Scheid, & Schmidt, 2005). There are at least four isoforms of NC, NC-A, NC-B, NC-C, and NC-D (Kurutz, Carvalho, & Nakagawa, 2003). The organic matrix of calcium oxalate kidney stones was found to have greater quantities of NC-C and NC-D isoforms than those of NC-A and NC-B isoforms found in people with normal kidney function (Kurutz et al., 2003).

3.1.3.3 Osteopontin

OPN is synthesised within the kidney and is present in the human urine. OPN is a negatively charged aspartic acid rich protein significant in bone mineralisation. Compared to nephrocalcin, OPN is more abundant in uric acid stones and with both calcium oxalate monohydrate and calcium dehydrate crystals (Kaneko et al., 2012). *In vitro* studies have shown that OPN may suppress the nucleation, growth, and aggregation of calcium oxalate crystals. In addition, OPN also inhibits the crystal adhesion to cultured epithelial cells (O'Brien et al., 1994). OPN may also act as a stone promoter because it has been found to increase crystal adhesion when bound to the surface of cells (Yamate, Kohri, Umekawa, Iguchi, & Kurita, 1998). A study by Nishio et al., (2000) reported reduced concentrations of OPN in urine from kidney stones patients compared to normal individuals (Nishio et al., 2000).

3.1.3.4 Urinary prothrombin fragment 1

UPTF1, is a potent inhibitor of calcium oxalate crystal aggregation and growth and reduces its adhesion to renal cells (Kumar, Farell, & Lieske, 2003; Worcester, Sebastian, Hiatt, Beshensky, & Sadowski, 1993). UPTF1 is a primary and minor constituent of both CaOx and uric acid crystals respectively (Aggarwal et al., 2013).

There is evidence that the amount of UPTF1 in kidney stones patients is significantly greater than in those of healthy subjects (Stapleton, Timme, & Ryall, 1998). The potent inhibitory effect of UPTF1 on CaOx crystallisation has been attributed to the Gla domain of the peptide found to be absent from its thrombin and F2 and both PT and F1 fragments (Grover & Ryall, 2002).

3.1.4 TYPES OF KIDNEY STONE

Generally, there are four types of kidney stones, with each type being distinguished by its own natural history and pathogenesis.

3.1.4.1 Calcium kidney stones

Calcium kidney stones account for 80% of all stone types. The majority of calcium stones are made up of calcium oxalate, with minimal calcium phosphate contribution (15%) (Xu, Zisman, Coe, & Worcester, 2013).

3.1.4.1.1 Calcium oxalate and phosphate kidney stones

Calcium oxalate kidney stones are formed when urine in the renal tubule is hyper-saturated by oxalate and calcium, which together forms crystals of dehydrated calcium oxalate (COD) and oxalic calcium monohydrated calcium oxalate (COM) (Lieske & Deganello, 1999). Calcium phosphate kidney stones are formed when urine pH is persistently alkaline and raised and are characterised by low serum bicarbonate concentration, hyperchloraemic metabolic acidosis, and hypokalaemia (Goldfarb, 2012). Various pathmechanisms including high calcium excretion in urine (hypercalciuria), high oxalate excretion in urine (hyperoxalouria), low citrate concentration in the urine (hypocitraturia), excessive concentrations of uric acid in urine (hyperuricosuria), reduced urine volume and abnormal urine pH are responsible for most calcium kidney stones formation. Systemic diseases including malabsorptive disorders (inflammatory bowel disease, severe diarrhoea or the resection of the intestine), and primary hyperparathyroidism also leads to formation of kidney stones, but to a lesser degree (Sakhaee, Maalouf, & Sinnott, 2012).

3.1.4.2 Uric acid stones

Uric acid stones account for 5% of total kidney stones (Han et al., 2015). Hyperuricosuria, characterised by the excessive production of uric acid increases the risk for the development of uric acid kidney stones (Ratkalkar & Kleinman, 2011). The causes of uric acid formation can be acquired, congenital and idiopathic, with idiopathic being the most common cause. A high purine diet, chronic diarrhoea, stressful physical exercise and inborn error of metabolism have not been implicated in the clinical and biochemical presentation of idiopathic uric acid kidney stones. Reducing the consumption of a purine rich diet can lead to marked improvement in urinary uric acid elimination (Sakhaee et al., 2012). The primary factors responsible for uric acid stone development in addition to hyperuricosuria include reduced urine volume and acidic urine (Dardamanis, 2013).

3.1.4.3 Struvite kidney stones

Struvite kidney stones, also known as infection stones, account for 15% of kidney stones (Dardamanis, 2013). Severe urinary tract infections (UTI), especially urease-producing bacteria (*Klebsiella*, *Pseudomonas* and *Proteus*) have been implicated as the cause as they increase the production of ammonia, carbonate and urinary pH in unison. The ensuing increased urine pH and ammonium concentrations promote the growth of struvite, either alone, or mixed with pre-existing calcium kidney stones (Zisman, Evan, Coe, & Worcester, 2015). Struvite kidney stones are three times more prevalent in women than men, since UTIs occur most in women (Flannigan, Choy, Chew, & Lange, 2014). Struvite stones may also form on infected UA or cystine as well as calcium stones, especially after surgical procedures and are likely to grow back after surgical removal because infected fragments of stones have been left behind (Trinchieri, 2014).

3.1.4.4 Cystine kidney stones

Cystine kidney stones only affect patients with cystinuria, an autosomal recessive disorder resulting from the abnormal renal tubule transport of dibasic amino acids, including cystine which in turn leads to high excretion of cystine in urine (Dawson & Tomson, 2012). It accounts for only 1% of patients with kidney stones in adults but is wide spread amongst children and adolescents with kidney stones (Chillaron et al.,

2010). Cystinuria is equally distributed among males and females, diagnosis can be made by finding typical hexagonal crystals in the urine (Fattah, Hambaroush, & Goldfarb, 2014; Mattoo & Goldfarb, 2008). Urinary tract infection and obstruction are common features of these stones, and is likely to reoccur every 1-4 years (Han et al., 2015).

3.1.5 KIDNEY DISEASE AND VITAMIN D SYNTHESIS

Vitamin D and PTH are the key regulators of calcium homeostasis, and the kidneys are the primary target organs affected by calcium regulatory hormones. The development of kidney disease is characterised by a decline in functional nephrons and glomerular filtration rate (GFR), which in turn decreases the renal excretion of phosphate, leading to increased serum calcium and phosphate concentrations (Gal-Moscovici & Sprague, 2007; Mozos & Marginean, 2015; Nigwekar, Bhan, & Thadhani, 2012). An increased serum phosphate concentration triggers the production of serum FGF-23, which downregulates the expression of renal 1 α -hydroxylase (CYP27B1) and upregulates 24-hydroxylase (CYP24A1) activity, resulting in reduced serum vitamin D concentrations (**Fig 3.1**) (Wolf et al., 2011). The reduced serum vitamin D concentrations cause a reduction in ionised serum calcium concentrations and an increase in serum PTH secretion (Secondary hyperparathyroidism) (Brito Galvao, Nagode, Schenck, & Chew, 2013). Under normal physiologic conditions, increased serum PTH concentrations upregulates the activity of CYP27B1, which causes an increase in vitamin D concentrations. However, in late CKD due to loss of functional tubules, increased serum PTH has been reported to be unable to regulate vitamin D synthesis (Brito Galvao et al., 2013)

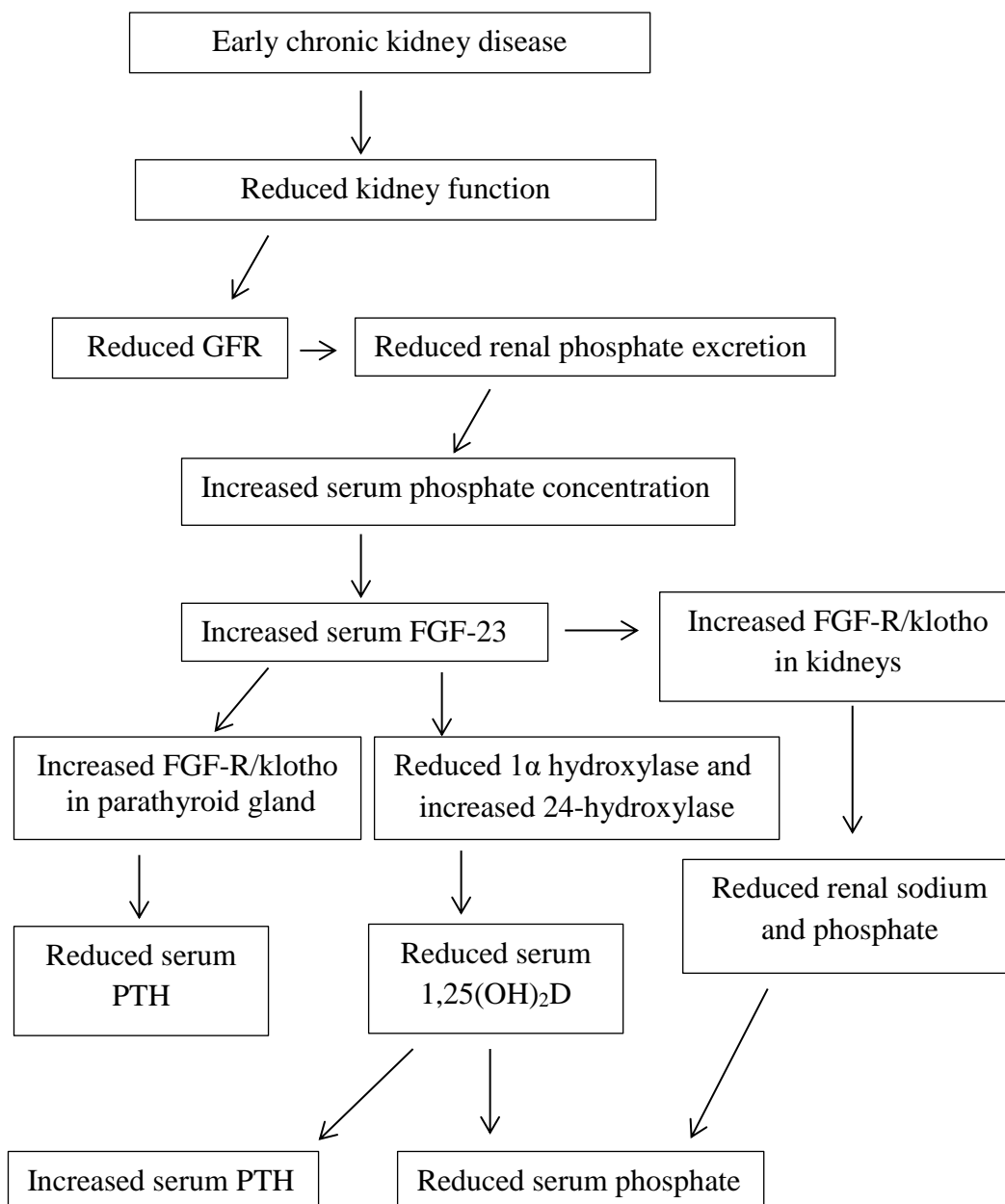


Fig 3.1 Role of reduced kidney function in vitamin D synthesis

3.1.5.1 Impaired vitamin D synthesis in kidney disease

Kidney disease patients have low vitamin D concentrations due to the increased secretion of FGF-23 from osteocytes (Hasegawa et al., 2010). The increase in FGF-23 as previously mentioned impairs vitamin D metabolism by reducing renal CYP27B1 mRNA expression and activity (Perwad & Portale, 2011). An increased circulating FGF-23 concentration in early CKD has been observed (Hasegawa et al., 2010). Rats with progressive CKD that were not treated with anti-FGF-23 neutralising antibody were found to have raised circulating FGF-23, PTH, and were vitamin D deficient and insufficient, thus demonstrating that higher FGF-23 leads to reduced vitamin D concentrations in early stage CKD. Low renal CYP27B1 expression, and raised renal CYP24A1 expression were also observed (Hasegawa et al., 2010). However, on administering FGF-23 antibodies, renal CYP27B1 mRNA expression increased, renal CYP24A1 mRNA expression decreased, and circulating vitamin D was restored to normal levels (Hasegawa et al., 2010), even though low serum vitamin D have not been consistently linked with decreases in renal CYP27B1 (Helvig et al., 2010).

Renal CYP27B1 activity may possibly be suppressed by acidic, hyperuricaemic, and uraemic milieu (Takahashi et al., 1998), as kidney abnormalities including high uric acid, are likely to decrease CYP27B1 expression (Vanholder, Patel, & Hsu, 1993). A study which investigated the effect of the uraemic toxin, uric acid on plasma concentrations of vitamin D in 9 renal failure patients found that uric acid, which is often raised in renal failure inhibits CYP27B1 activity and the synthesis of vitamin D (Vanholder et al., 1993).

Increased circulating FGF-23 in kidney disease also promotes the transcription of CYP24A1 which leads to an increase in 25(OH)D and 1,25(OH)₂D catabolism (Jones et al., 2012; Petkovich & Jones, 2011). Given the effects of FGF-23 on CYP27B1 and CYP24A1 transcription, observations from studies that have assessed the significance of CKD on renal CYP27B1 and CYP24A1 expression and activity have shown conflicting results. A study in nephrectomised rats, which investigated the changes in mRNA levels of CYP27B1 and CYP24A1, showed significant increase in CYP27B1 and decrease in CYP24A1 mRNA expression (Takemoto et al., 2003). Another study in a rat model aimed at evaluating whether CYP24A1 and CYP27B1 expression is altered in uraemia and in normal, adenine-induced CKD, as

well as in renal biopsy tissue from patients with kidney disease reported high circulating CYP24A1 mRNA expression in diseased compared to healthy renal tissue (Helvig et al., 2010). Results from this study suggested that the increase may be induced by factors associated with the uraemic state, which may have significant effects on vitamin D status and possibly affect tissue responsiveness to vitamin D therapy. This study also reported increased renal CYP27B1 mRNA and protein concentrations in the adenine-induced CKD rats, suggesting that the observed reductions in vitamin D concentrations were not due to changes in CYP27B1 expression and activity (Helvig et al., 2010).

The reduced vitamin D synthesis observed in kidney disease may also be due to disruptions in 25(OH)D transport to CYP27B1 in the renal proximal tubule by megalin and cubulin, as megalin expression in renal tubules declines in kidney disease (Bosworth & de Boer, 2013). It has been observed that less 25(OH)D is filtered following diminishing GFR, thus, leaving little 25(OH)D available for reabsorption into the proximal tubules of the kidneys. The decline in the expression of megalin in kidney disease may hinder filtration of 25(OH)D and may cause albuminuria (Takemoto et al., 2003). Due to the ensuing albuminuria presence in kidney disease, the filtered 25(OH)D which normally goes through proximal tubular reabsorption may be lost in urine (Thraillkill, Jo, Cockrell, Moreau, & Fowlkes, 2011). Albuminuria may also be accompanied by high urinary loss of VDBP, leading to increased renal loss of all vitamin D metabolites (Andress, 2005). In non-haemodialysis patients with CKD stages 1 to 5, Gonzalez *et al.* found that 10 of 11 patients with nephrotic albuminuria had remarkably low serum concentrations of 25(OH)D (Gonzalez, Sachdeva, Oliver, & Martin, 2004). Even though 25(OH)D substrate is well absorbed into the proximal tubules, conversion to 1,25(OH)₂D may be hindered by diminished CYP27B1 activity (Levin et al., 2007), or by the reduced metabolic capacity of the proximal tubular cells due to oxidative stress or a reduction in functioning nephrons (Granata et al., 2009). Furthermore, the increased PTH concentrations associated with low vitamin D concentrations depletes vitamin D body stores by promoting 24, 25-dihydroxylases (Shimada, Hasegawa, et al., 2004).

Several factors independent of renal function have been implicated for vitamin D deficiency in kidney disease (Nigwekar et al., 2012). Patients with kidney disease are likely to have reduced sun exposure (Hu et al., 2011) and impaired skin synthesis of

vitamin D after UVB irradiation, despite having normal epidermal content of 7DHC (Nigwekar, Tamez, & Thadhani, 2014). Advancing kidney disease is associated with diverse cutaneous abnormalities including hyperpigmentation (Udayakumar et al., 2006). The hyperpigmentation is caused by increasing melanin, in the epidermis due to an increase in the uraemic toxin known as non-dialysable middle molecules (Robinson-Bostom & DiGiovanna, 2000). The progressive retention of this uraemic toxin in the epidermis that absorbs UVB irradiation resulting in hyperpigmentation may be responsible for the reduced vitamin D synthesis in kidney disease (Abdelbaqi-Salhab, Shalhub, & Morgan, 2003; Kim & Kim, 2014). Furthermore, intake of foods rich in vitamin D such as dairy products and egg yolk are reduced in patients with kidney disease because of the dietary restrictions advised to kidney disease patients. The purpose of the diet restrictions is to maintain the levels of electrolytes, minerals, and fluids and to limit the accumulation of waste products in the body of kidney disease patients (Cuppari & Garcia-Lopes, 2009). Dysfunctional kidneys have also been reported to impair gastrointestinal vitamin D absorption (Nigwekar et al., 2012). Kidney disease is also characterised by a gradual loss of VDR in the parathyroid gland resulting in vitamin D resistance (Fukuda et al., 1993). In addition, low concentrations of vitamin D further lead to impaired binding of vitamin D to VDR, as well as in the binding of the vitamin D/VDR complex to the VDRE in kidney dysfunction compared with normal kidney function (Patel, Ke, Vanholder, Koenig, & Hsu, 1995). This could mean that vitamin D may not be able to exert its primary biological effects on tissues and organs in kidney disease or VDR activity is reduced in kidney disease.

3.1.6. KIDNEY STONE, VITAMIN D AND CVD RISK

There is impaired renal function in kidney stone disease (Zisman, Evan, Coe, & Worcester, 2015), thus kidney stone disease is a risk factor for CKD, as it has been reported that the risk for developing CKD among kidney stone patients was 50-67% higher, compared to controls with normal kidney function (Rule et al., 2009). CKD is characterised by low serum 25(OH)D concentrations and increased CVD risk (Kim & Kim, 2014). The increased risk of CKD in kidney stones patients would predispose them to increased CVD risk (Rule et al., 2010). CVD risk is higher in kidney disease patients compared to patients with normal kidney function. A study in 4,564 kidney stone disease formers and 10,860 age and gender matched controls

showed that kidney stone disease formers had increased risk of myocardial infarction compared with the controls (Rule et al., 2010). Kidney stone disease formers have also been reported to be more likely to have baseline diagnosis of dyslipidaemia, hypertension, diabetes and obesity (Hamano et al., 2005). The increased CVD risk in kidney stone disease patients may be attributed to sharing common pathomechanisms with CVD (Rule et al., 2010). Uraemia, characterised by fluid, electrolyte and hormone imbalance, as well as metabolic anomalies, develops with the decline of kidney function (Odabaei, Kaysen, & Ananthakrishnan, 2014). Uraemia has been reported as the established link between CVD and kidney disease, as it increases CVD burden (Fahal, 2014). The increase in CVD risk in kidney disease, may be due to several risk factors including impaired calcium and phosphate, low serum 25(OH)D concentrations, elevated PTH, renal inflammation, endothelial dysfunction, increased oxidative stress and dyslipidaemia (Brunet et al., 2011; Nakano et al., 2010).

In the kidneys, besides regulating renal inflammation and fibrosis (Wang et al., 2006), vitamin D exerts important renal reclamation of filtered 25(OH)D, important in maintaining sufficient serum/plasma 25(OH)D concentrations. Sufficient serum 25(OH)D concentration is important for the pleiotropic actions of vitamin D in endothelial cells, cardiac and vascular smooth muscle, mediated through the autocrine/paracrine pathways that impart the health of these tissues (Brito Galvao et al., 2013; Hur et al., 2009). However, these important cardioprotective functions of vitamin D are lost with declining kidney function leading to reduced serum 25(OH)D concentrations and increased CVD risk (Kim & Kim, 2014).

3.1.6.1 Vitamin D, impaired calcium-phosphate product and CVD

As previously mentioned, vitamin D together with PTH, acts to maintain serum phosphate and calcium concentrations within normal limits, thus sustaining an optimal calcium-phosphate product for bone mineralisation without deposition in soft tissues (Moe, 2008). Since calcium and phosphorus are critical elements in vascular calcification, the association between vitamin D deficiency and CVD risk in human kidney disease may in part be mediated through disruptions in calcium-phosphate homeostasis (Moe, 2008). The pathological pathways responsible for kidney stone formation are also involved in coronary artery calcification or calcium (CAC) that

leads to atherosclerosis (Yiu, Callaghan, Sultana, & Bandyopadhyay, 2015). Increased serum calcium-phosphate product may result in kidney stone formation (London et al., 2007) and may as well cause the precipitation and deposition of calcium and phosphate in arteries resulting in arterial calcification (Covic, Voroneanu, & Goldsmith, 2010). A study which investigated the distribution of calcium-phosphate in all kidney stone types, found that whilst increased serum calcium concentrations stimulated the mineralisation of VSMC, increased serum phosphate concentrations enhanced the mineralisation (Parks, Worcester, Coe, Evan, & Lingeman, 2004). Increased serum calcium and phosphate product have also been reported to facilitate calcification in CKD (Cozzolino, Brancaccio, Gallieni, & Slatopolsky, 2005). Based on the hypothesis that vitamin D regulates calcium and phosphate product, inverse associations between vitamin D status and arterial calcifications ($r = -0.18$; $P = 0.024$) were reported in a cross section of 173 people at risk for coronary heart disease (Watson et al., 1997). Serum FGF-23 concentrations are also involved in arterial calcification, as vitamin D induces FGF-23 secretion and is in turn inhibited by increased FGF-23 concentrations (Zisman & Wolf, 2010). Additionally, an increased circulating FGF-23 concentration is associated with arterial calcification in patients with all stages of CKD, even though its function is not well understood (Shimada et al., 2010).

Associations between coronary artery calcification determined by coronary artery calcium (CAC) in recurrent kidney stone have been reported in a USA multi-ethnic study in 6,814 men and women free of CVD from 2010-2012 (OR 1.80, 95% CI 1.22-2.67) (Hsi et al., 2015). Vascular calcification in kidney disease may also result from the imbalance between promoters and inhibitors of kidney stone formation (Shanahan, 2005). Kidney stone patients compared with non-stone forming people, are more prone to vascular calcifications due to decreased concentrations of uropontin, a glycoprotein that inhibits calcification in urine (Kleinman, Wesson, & Hughes, 2004). Hence, it may be possible that vascular calcification is present in kidney stone patients, particularly calcium oxalate kidney stone patients, as mice deficient in this glycoprotein have been reported to develop calcium oxalate stones after hyperoxaluria was induced (Wesson et al., 2003). It was hypothesised that increased plasma OPN concentrations may be associated with the presence of aortic valve calcification and stenosis. A study in 30 elderly (> 65 years) patients with

(n=23) and without (n=7) aortic stenosis, showed that patients with aortic stenosis had higher osteopontin levels compared with patients without aortic stenosis (652.2 ± 218.7 vs 379.7 ± 159.9 ng/mL, $P < .01$) (Yu et al., 2009).

Furthermore, the constant exposure of VSMCs to increased concentrations of calcium and phosphate directly induce the osteogenic transformation of VSMCs, initiating and promoting vascular calcification. This conversion is mediated by the activation of osteogenic/chondrogenic gene expression including Runt-related transcription factor 2 (RUNX2), alkaline phosphatase (ALP), osterix and OPN, and the subsequent downregulation of smooth muscle lineage gene expression (SM α actin, SM22 α) (Shanahan, Crouthamel, Kapustin, & Giachelli, 2011). In addition to increased calcium and phosphate concentration, the increased production of ROS observed in calcium oxalate kidney stones have also been implicated in the osteogenic transformation of VSMC by also regulating RUNX2 transcription (Byon et al., 2008).

3.1.6.2 Vitamin D, PTH and CVD risk in kidney disease

Vitamin D impart benefits in kidney disease patients by lowering PTH concentration (Slatopolsky & Delmez, 1996). The FGF-23-mediated decrease in vitamin D synthesis stimulates PTH secretion and initiates secondary hyperparathyroidism (Seiler, Heine, & Fliser, 2009), which occurs prior to the development of phosphaturia observed in kidney disease (Folsom et al., 2014). PTH functions to regulate vitamin D, calcium and phosphate concentrations and to maintain skeletal integrity, as it stimulates the synthesis of vitamin D following a decline in vitamin D concentrations (Hagstrom et al., 2009; Hu et al., 2011). PTH performs extensive roles in health and diseases involving different organs and systems including the CV system. The presence of PTH receptors and PTH- related peptides in the myocardium and vasculature suggest a potential role of PTH in CVD (Potthoff et al., 2011). A large observational study of a US cohort of CKD patients have shown that low serum 25(OH)D and elevated serum PTH concentrations are common with reduced kidney function (Levin et al., 2007). A meta-analysis of 17 observational and 5 RCT studies, showed significant improvement in serum/plasma 25(OH)D concentrations (MD 24.1 ng/ml, 95% CI 19.6 to 28.6) and an associated reduction in serum/plasma PTH concentration (MD 41.7 pg/ml, 95% CI 55.8 to 27.7) amongst observational studies.

A significant improvement in serum/plasma 25(OH)D concentrations (MD 14 ng/ml, 95% CI 5.6 to 22.4), with reductions in serum/plasma PTH concentrations (MD 31.5 pg/ml, 95% CI 5.7 to 6.1) were reported among the RCTs in CKD patients (Kandula et al., 2011). Similarly, in 14,289 unselected US CKD stages 3-5 patients, increased serum 25(OH)D concentrations, resulted in low serum PTH concentrations (Ennis, Worcester, Coe, & Sprague, 2016). These observations suggest that low serum 25(OH)D concentrations observed in kidney disease will subsequently lead to increased serum PTH concentrations.

Studies have also shown that severe increase in PTH concentration is associated with increased CV events including, hypertension, cardiac hypertrophy and myocardial infarction (Saleh, Schirmer, Sundsfjord, & Jorde, 2003; Snijder et al., 2007). The findings from a meta-analysis of 15 observational human studies conducted between 1947 to October 2012, found an association between high PTH and a 1.45 fold increase in CVD events incidence compared with low levels of PTH HR 1.45 (1.24-1.71; $P < .001$) (van Ballegooijen, Reinders, Visser, & Brouwer, 2013). A cross section of 558 men with high serum PTH had a significantly higher risk of calcification of the abdominal aorta compared with men in the low serum PTH group (OR 4.03, CI 1.50–10.83, $P = 0.01$) (Buizert et al., 2013).

Elevated serum PTH concentrations have also been implicated in lipid abnormalities, as it promotes atherosclerosis in kidney disease. Associations have been reported between secondary hyperparathyroidism and the increased intima media thickness of both carotid and femoral arteries in CKD patients undergoing haemodialysis (Nishizawa, Shoji, Kawagishi, & Morii, 1997). Findings from this study showed that hyperparathyroidism adversely inhibits hepatic triglyceride lipase, a lipid-regulating enzyme that performs essential functions in HDL-C metabolism. However, a large community study of Whites and African Americans that measured PTH in stored serum samples from 1990 to 1992 did not demonstrate that elevated serum PTH concentration was associated with any CVD outcome marker (Folsom et al., 2014).

Potential explanations for the association between high serum PTH concentration and CV mortality in kidney disease include the following: Firstly, the relationship between PTH and atherosclerosis is mediated through its association with endothelial dysfunction, raised aortic pulse pressure and decreased large artery elasticity

(Bosworth et al., 2013). Additionally, PTH exerts detrimental effects on the myocardium by inducing left ventricular hypertrophy, cardiac calcification and fibrosis (Andersson, Rydberg, & Willenheimer, 2004; Ellero et al., 2010). Finally, higher PTH is associated with inflammatory markers as it induces cytokine release from VSMCs and lymphocytes (Martin-Ventura et al., 2003). Thus, the interaction between high PTH levels due to vitamin D deficiency and cardiovascular mortality in kidney disease is complex.

3.1.6.3 Vitamin D, inflammation and endothelial dysfunction in kidney disease

Kidney diseases are characterised by renal inflammation, suggesting an important association between kidney disease and CVD (Tripepi, Mallamaci, & Zoccali, 2005). Patients with acute renal inflammation determined by urinary MCP-1 have been reported to have low serum 25(OH)D concentrations (Zehnder et al., 2008), which triggers both vascular and systemic inflammation (Yadav, Banerjee, Lal, & Jha, 2012). A cross-sectional study in 101 stage 4-5 non-dialysis CKD patients and 40 healthy controls found higher mean (\pm SD) serum hs-CRP concentrations of 90.7 ± 5.8 vs. 50.1 ± 8.6 μ g/mL, ($P \leq 0.0001$) with lower mean (\pm SD) serum 25(OH)D concentrations 44.8 ± 4.8 vs 67.3 ± 8.8 nmol/L, ($P \leq 0.0001$) in the CKD patients compared with controls. The serum hsCRP concentration was inversely associated with serum 25(OH)D concentrations ($r = -0.2$, $P = 0.047$) in CKD patients (Yadav et al., 2012).

Uraemia, which is observed in kidney disease causes endothelial dysfunction that downregulates NO synthesis, reducing the vasodilatory property of the endothelium resulting in early atherosclerosis (Brunet et al., 2011; Nakano et al., 2010). High occurrence of atherosclerosis in renal disease has been reported (Tonelli et al., 2001), a cross-sectional study of 126 autopsy samples showed that the incidence of advanced atherosclerotic lesions increased gradually as kidney function determined by eGFR decreased (33.6%, 41.7%, 52.3%, and 52.8% for eGFRs ≥ 60 , 45-59, 30-44, and <30 mL/min/1.73 m respectively; $P = 0.006$) (Nakano et al., 2010). The uraemic toxin indoxyl sulphate, derived from dietary proteins has been shown to cause endothelial dysfunction by inducing leukocyte-endothelial interactions through the up-regulation of E-selectin (Ito et al., 2010). The assessment of the *in vivo* effect of indoxyl sulphate in nephrectomised CKD model mice demonstrated that indoxyl

sulphate magnified leukocyte-endothelial interactions through the up-regulation of E-selectin, probably via the JNK- and NF- κ B-dependent pathway (Ito et al., 2010).

Additionally, another uraemic toxin, asymmetric dimethylarginine (ADMA), an inhibitor of NO synthase has been linked to reduced kidney function and increased atherosclerotic events. The study investigating the relationship between plasma ADMA and markers of atherosclerosis including carotid intima-media thickness (IMT), soluble vascular cell adhesion molecule-1 (sVCAM-1) in 93 patients with mild-to-moderate renal failure, found that kidney function was inversely associated with increased plasma ADMA ($r = -0.342$, $P = 0.023$). Increased plasma ADMA was in turn positively associated with carotid intima media thickness ($r = 0.444$, $P < 0.0001$) and plasma sVCAM-1 concentration ($r = 0.242$, $P = 0.022$) (Nanayakkara et al., 2005).

Low vitamin D concentrations observed in kidney disease patients have been shown to stimulate endothelial dysfunction resulting in early atherosclerotic vascular disease (Chitalia, Recio-Mayoral, Kaski, & Banerjee, 2012). A study which assessed the relationship between serum 25(OH)D concentrations and endothelial function determined by FMD in 50 non-diabetes patients with mild to moderate CKD, found that low serum 25(OH)D (≤ 37.5 nmol/L) concentration were associated with low FMD ($r = 0.44$, $P = 0.001$) compared to patients with 25(OH)D > 37.5 nmol/L, mean (\pm SD), $4.4 \pm 2.5\%$ vs. $2.5 \pm 1.6\%$, ($P = 0.007$) (Chitalia et al., 2012). Another study in 117 non-dialysis CKD patients showed that FMD was reduced in vitamin D deficient and insufficient compared to the vitamin D sufficient groups, with the lowest value observed in the vitamin D-deficient group (Zhang et al., 2015). This study also demonstrated that serum sVCAM-1 and sE-selectin were higher in the vitamin D deficient and insufficient groups compared to the vitamin D sufficient group, with the highest value been observed in the vitamin D deficient group. There was a positive association between FMD and serum 25(OH)D concentration ($r = 0.556$, $P < 0.001$) and inverse associations between serum 25(OH)D concentrations with serum sVCAM-1 ($r = -0.549$, $P < 0.001$) and sE-selectin concentrations ($r = -0.360$, $P < 0.001$) (Zhang et al., 2015). These observations suggest that low serum 25(OH)D concentrations are a significant factor linking CKD to CVD (Pilz et al., 2011).

3.1.6.4 Vitamin D, oxidative stress and CVD risk in kidney disease

The release of ROS, resulting in oxidative stress in kidney dysfunction, has been implicated as the primary contributor of CVD in renal disease (Khan, 2014). Calcium oxalate kidney stones are excreted or retained in different parts of the urinary tract during their formation, and this retention usually obstructs the renal tubules (Joshi, Peck, & Khan, 2013). The obstructed renal tubules, in turn disrupt cellular functions by damaging cell membranes, and releasing both lipid mediators and ROS (Joshi et al., 2013). The release of ROS results in oxidative stress that in turn lead to renal injury, inflammation, decreased and impaired renal function and end stage renal disease (Joshi et al., 2013). Oxidative stress is responsible for the production of advanced glycation products and endothelial dysfunction (Sebekova et al., 2015), as it depletes NO signalling pathway resulting in the conversion of NO to peroxynitrite, a potent oxidant that oxidises anti-oxidants such as tetrahydrobiopterin. Absence of tetrahydrobiopterin leads to endothelial NOS uncoupling, which in turn modifies this enzyme from being vasoprotective to being an oxidative stress enzyme (Joshi et al., 2013). Reduced NO may intensify inflammation and activate NF- κ B pathway, which mediates severe inflammation and fibrogenesis through the modulation of cytokines, chemokines and adhesion molecules including IL-6, MCP-1, and TNF- α expression. The severe inflammation results in the expression of adhesion molecules such as E-selectin on the endothelium and the proliferation of VSMC (Guijarro & Egido, 2001; Lassegue et al., 2001). Oxidative stress also increases uric acid production by the endothelium and kidneys that alters renal function. Increased uric acid production promotes severe inflammatory response in renal epithelial cells, whilst decreasing NO synthesis and activating NF- κ B, thus, increasing the risk of CVD (Gersch et al., 2008; Han et al., 2007; Umekawa, Chegini, & Khan, 2003).

There is evidence that vitamin D deficiency increases the risk for diseases that are caused by oxidative stress as is observed in kidney disease, although such studies that reported a relationship between vitamin D status and oxidative stress to the best of my knowledge are absent in renal disease patients. A study in 1488 school children ages 7 to 11 years aimed at investigating 25(OH)D concentrations and its association with oxidative stress determined by superoxide dismutase found that the low serum concentration 25(OH)D <50nmol/L group had lower mean (\pm SD) concentrations of serum superoxide dismutase compared with those in the vitamin D

sufficient group, 95.38 ± 12.22 vs. 127.62 ± 15.98 U/mL, ($P < 0.001$) (Zhang *et al.*, 2014).

3.1.6.5 Vitamin D, Dyslipidaemia and CVD risk in kidney disease

Dyslipidaemia contributes to the associations between kidney disease and the onset of atherosclerosis (Moradi, Vaziri, Kashyap, Said, & Kalantar-Zadeh, 2013), as it is characterised by low plasma HDL, high TAG, TC, LDL-C and lipoprotein A concentrations (Hu, Lu, Hu, & Du, 2009; Vaziri & Moradi, 2006). The hypercholesterolemia observed in kidney dysfunction has been ascribed to increased hepatic synthesis of apolipoprotein B (apoB), whilst increased triacylglycerol concentrations results from impaired catabolism of TAG-rich lipoproteins (Vega, Toto, & Grundy, 1995). Dyslipidaemia is not only involved in increased CVD risk, it also accelerates the development of kidney dysfunction (Ritz & Wanner, 2008). Additionally, HDL-C performs essential roles in maintaining the integrity of the vasculature (Barter *et al.*, 2007), and this function differs with varying clinical conditions (Sorrentino *et al.*, 2010). The primary vasoprotective property of HDL-C, is its role in reverse cholesterol transport, which is the recovery and transport of excess cholesterol from extrahepatic tissues for clearance in the liver (Vaziri & Norris, 2011).

The cholesterol clearance capacity of HDL-C has been reported to be decreased in patients with kidney disease compared to healthy individuals (Yamamoto *et al.*, 2012). Kidney disease which is linked with advanced glycation, oxidative stress, inflammation and uraemic toxins can alter HDL-C function by changing its lipid and protein composition (Holzer *et al.*, 2011). Kidney dysfunction also stimulates posttranslational changes of HDL's protein cargo (Zheng *et al.*, 2004) by interacting with the HDL-C particle non-covalently (Speer *et al.*, 2013). A dysfunctional HDL-C loses its ability to reverse cholesterol transport and its cardio-protective functions. Several studies have reported dyslipidaemia in kidney stone formers. A retrospective analysis of a random cohort of 60,000 patients found low concentrations of HDL-C, 47 versus 50 mg/dL, ($P = 0.001$) to be associated with kidney stone compared with non-kidney stone patients (Masterson *et al.*, 2015). A study aimed at evaluating the lipid profile of 49 kidney stone patients compared with the controls showed that mean (\pm SD) TC 218.6 ± 45.7 vs. 195.4 ± 36.4 mg/dL, ($P < 0.05$) and mean (\pm SD)

TAG 243.6 ± 200.2 vs. 127.5 ± 63.8 mg/dL ($P < 0.01$) concentrations were significantly higher in kidney stones patients compared with the control (Inci, Demirtas, Sarli, Akinsal, & Baydilli, 2012). The study aimed at assessing the association between dyslipidaemia in 655 stone formers and 1965 controls found out that stone formers had significantly higher mean (\pm SD) TAG (166.9 ± 80.2 vs 138.0 ± 67.2 mg/dL, ($P < 0.001$) with low mean (\pm SD) HDL-C concentrations 48.1 ± 12.3 vs. 52.5 ± 13.1 mg/dL, ($P < 0.001$) compared with controls (Kang et al., 2014). One study which explored the regulators of cholesterol synthesis that results in lipid accumulation in human diabetic nephropathy, found significant downregulation of cholesterol efflux genes and APO E and the activation of most lipoprotein receptors (Herman-Edelstein, Scherzer, Tobar, Levi, & Gafter, 2014). These alterations in cholesterol metabolism gene expression are responsible for mediating increased cholesterol influx, whilst impairing cholesterol efflux and this is the possible mechanism for renal lipid accumulation in renal dysfunction (Herman-Edelstein et al., 2014). HDL-C dysfunction begins in early CKD and continues as renal function reduces, the endothelial properties of HDL-C as well as its cholesterol efflux capacity are potently impaired in children with early CKD (Shroff et al., 2014). The HDL-C alterations were also observed in early CKD without any underlying inflammatory, diabetes, or coronary artery disease, suggesting that CKD itself changes HDL into a toxic pro-atherogenic particle that facilitates endothelial dysfunction (Shroff et al., 2014).

Most studies in kidney stone disease patients have been unable to demonstrate an association between serum or plasma 25(OH)D concentration and lipid profile markers. However, there is evidence that vitamin D is associated with dyslipidaemia in kidney disease (Khajehdehi, 2000). A significant inverse association has been reported between plasma 25(OH)D and triacylglycerol concentrations ($r = -0.14$, $P = 0.001$) in 495 non-diabetic CKD patients (Seiki et al., 2012).

3.1.7. Prevalence of vitamin D deficiency in kidney stone patients

Serum 25(OH)D concentration has been reported to be significantly reduced in chronic estimated GFR reduction compared with those in participants with normal kidney function (Chonchol & Scragg, 2007). A case control study in 884 idiopathic calcium kidney stones patients and 967 controls, found that the prevalence of vitamin

D deficiency (serum 25(OH)D < 50 nmol/L) was 56% in the kidney stones formers and 44% in controls with mean concentration of 45 nmol/L vs. 57.5 nmol/L, ($P = 0.02$) respectively (Ticinesi et al., 2016). Similarly, Giron-Prieto *et al.*, found a 28% prevalence of vitamin D deficiency (25(OH)D < 50 nmol/L) in calcium stone-forming patients compared to 15.7% in non-formers by 64.3 vs. 71 nmol/L, ($P = 0.02$). Additionally, a retrospective study aimed at assessing the prevalence and metabolic abnormalities of 101 vitamin D deficient and insufficient (25(OH)D < 50 nmol/L and 52.5-72.5 nmol/L, respectively) patients presenting with kidney stones found 33.7% and 46.5% of stone formers were vitamin D deficient and insufficient respectively (Elkoushy, Sabbagh, Unikowsky, & Andonian, 2012). Another retrospective review of 169 kidney stone formers, carried out in Rhode Island and Boston stone clinics also discovered that 18.9% and 34.9% were vitamin D deficient and insufficient (25(OH)D < 50 nmol/L and 52.5-72.5 nmol/L) respectively (Eisner, Thavaseelan, Sheth, Haleblan, & Pareek, 2012).

Furthermore, a third of 236 patients with recurrent kidney stone have also been reported to have vitamin D insufficiency (25(OH)D < 50 nmol/L) (Pipili & Oreopoulos, 2012). In contrast, one study using data from the third National Health and Nutrition Examination Survey (NHANES) to determine the association between serum 25(OH)D concentration and prevalent kidney stone disease in 16,286 men and women aged 18 years or older. The study found no significant difference in serum 25(OH)D concentrations amongst kidney stone patients compared to people with normal kidney function (Tang, McFann, & Chonchol, 2012).

3.1.8 STUDY RATIONALE

Data from observational and experimental studies have shown that patients with kidney disease often have low concentrations of serum 25(OH)D and increased CVD risk. Due to the loss of nephron mass and reduced GFR in kidney disease, calcium and phosphate homeostasis are disrupted. This imbalance stimulates the osteocytes to secrete more FGF23 in order to maintain homeostasis, as a result the increased FGF-23 leads to reduced vitamin D synthesis as FGF23 inhibits and facilitates both CYP27B1 and CYP24A1 expression and activity respectively. Reduced vitamin D concentrations would in turn increase PTH concentrations implicated in CVD events.

Additionally, the increase in the uraemic toxin uric acid also reduces vitamin D synthesis, as well as VDR and the vitamin D/VDR interaction, thus the beneficial effects vitamin D exerts through its autocrine and paracrine mechanism through increased VDR activity would be reduced in the VSMC, endothelium and cardiomyocytes in kidney disease which may in turn increase CVD risk. Given the multiple reported effects of vitamin D on PTH, inflammation, endothelial dysfunction and other CVD risk factors, we hypothesise that kidney stone patients would be vitamin D deficient and this deficiency would be associated with specific CVD outcome measures. Furthermore, evidence about the potential association between serum 25(OH)D concentrations and CVD markers in kidney stone disease patients, particularly calcium oxalate kidney stone patients are so far lacking. Hence, the need for a cross-sectional study establishing such relationship.

3.1.8.1 RESEARCH PURPOSE

To determine the prevalence of vitamin D deficiency and CVD risk in calcium oxalate kidney stone patients.

3.1.8.2 OBJECTIVES

- To assess the vitamin D status in male and female patients with calcium oxalate kidney stone.
- To assess individual CVD risk factors in male and female patients with calcium oxalate kidney stone.
- To investigate the association between vitamin D status and specific CVD risk factors in male and female calcium oxalate kidney stone patients.

3.1.8.3 HYPOTHESES

H₁: Male and female calcium oxalate kidney stone formers will be vitamin D deficient with serum 25(OH)D < 50 nmol/L.

H₀: Male and female calcium oxalate kidney stone formers will not be vitamin D deficient with serum 25(OH)D < 50 nmol/L.

H₁: Male and female calcium oxalate kidney stones formers will be at increased risk of CVD.

H₀: Male and female calcium oxalate kidney stone formers will not be at an increased risk of CVD

H₁: There will be an association between vitamin D status and the CVD risk biomarkers in male and female calcium oxalate kidney stone patients

H₀: There will not be an association between vitamin D status and the CVD biomarkers male and female calcium oxalate kidney stone patients.

3.2 STUDY DESIGN FOR KIDNEY STONES STUDY

This is a non-interventional cross-sectional study of a cohort of kidney stone disease patients, about to undergo shock wave lithotripsy (SWL) treatment from the local hospital (Wrexham Maelor and Glan Clywd Hospital, United Kingdom). Ethical approval for this study was sought from the Wales Research Ethics Service (REC) 4 Committee (REC4:12/WA0117). This study retrospectively analysed stored baseline serum samples collected from 24 (15 males and 9 females) patients diagnosed with calcium oxalate kidney stones, patients were recruited after written informed consent from September 2013 to May 2015. Those who had a pre-existing clinical condition or are receiving treatment for any other ailment were excluded from the study. The data collected includes age, gender and the medications they were taking.

3.2.1. Blood sampling

The serum samples prior to SWL were collected in serum separator tubes, stored at -80°C until analysed. Blood sampling for this study was performed by a research student.

3.2.2 Biochemical measurement

Material Safety Data sheets and COSHH information were adhered to for each analyte. Prior to the experiments all reagents, microplate wells, and samples were brought to room temperature according to appropriate kit instruction booklet. To avoid cross contamination, pipette tips were changed during each standard preparation, and screw caps were also not interchanged. Multiple freeze thawed samples were not used, and all ELISAs were performed in duplicate. To ensure accurate results, only calibrated pipettes were used, kits were used before expiration date and reagents were not mixed with those from other sources or lots. Plasma samples which had concentrations that exceeded the highest standard were further diluted and repeated.

Prior to use of each ELISA kit, a QC was run 8 times to obtain the intra-assay CV, and the inter-assay CV was obtained from commercial control assay. These CVs were calculated using the formula:

$$CV = \frac{S.D}{Mean} \times 100$$

3.2.2.1 Total serum 25(OH)D concentration determination

Plasma total 25(OH)D was measured using mini VIDAS® (BioMérieux, Hampshire, UK), a compact multi-parametric automated immuno-analyser based on the enzyme linked fluorescent assay principles. The 25(OH)D total assay kit contained a ready to use solid phase receptacle (SPR) and stabiliser strips coated with vitamin D. Plasma (100 µL) was pipetted into each SPR well and inserted in machine. After 40 minutes run time, 25(OH)D concentration was obtained. The intra and inter assay CV were 2.0 % and 7.3 % respectively.

3.2.2.2 Serum calcium concentration determination

Calcium concentration was determined using a calcium colorimetric detection kit. Prior to the assay serum samples were diluted in a 1:1 ratio (25 µL sample + 25 µL deionised water). Calcium standard was diluted by adding 10 µL of the 500 mM standard to 990 µL deionised water to produce a stock solution which was further diluted serially. Each of the six serial dilutions was brought to a final volume of 50 µL per well by adding deionised water.

Following sample and reagent preparation, 90 µL of chromogen reagent was pipetted into each well containing the standard, control and sample. Following this, 60 µL of calcium assay buffer was pipetted into each well, plate was mixed thoroughly and incubated for 5-10 minutes at room temperature. Calcium concentration was determined at a wavelength of 575nm and obtained concentrations were multiplied by the corresponding dilution factor (see appendix for example standard curve. The intra and inter assay CV was 4.3% and 9.8% respectively.

3.2.2.3 Serum PTH concentration determination

A solid phase direct sandwich ELISA kit (Calbiotech, Spring valley, USA) precoated with streptavidin, quantitatively determines PTH concentration in plasma. Prior to the assay, 25 mL of 1x wash buffer was added to 475 mL deionised water to yield a final volume of 500 mL. Stock enzyme conjugate (0.5 mL) was added to assay diluent (9.5 mL).

Following reagent preparation, standard, blank, control and samples (25 µL each) were pipetted into appropriate wells. Anti-PTH-biotin reagent (50 µL) and anti-PTH-

HRP conjugates (50 μ L) were pipetted into all wells respectively. The microplate was covered with adhesive strip and incubated at room temperature on a plate shaker set at 500-600 rpm for 90 minutes.

Following incubation, wells were washed 4 times with 300 μ L wash buffer, decanted and blotted dry on clean absorbent paper towels. Following the wash step, 100 μ L of TMB substrate was pipetted into all wells, and incubated for 15 minutes at room temperature. Stop solution (50 μ L) was pipetted into each well and optical density was determined within 15 minutes at a wavelength of 450nm (primary) and 630nm (reference). PTH concentrations were obtained from a standard curve (see appendix 9 for example standard curve). Intra and inter assay CV were 4.7% and 2.6% respectively.

3.2.2.4 Serum hs-CRP concentration determination

A solid phase direct sandwich ultra-sensitive ELISA kit (Calbiotech, Spring valley, USA) precoated with anti-CRP monoclonal antibody, quantitatively determined hs-C reactive protein concentration in plasma. Prior to the assay, samples and controls were diluted 1:100 fold by adding 5 μ L of sample to 495 μ L sample diluent. Wash buffer (25 mL) was added to 475 mL deionised water to yield a final volume of 500 mL.

Following reagent preparation, 10 μ L standard, blank, control and sample were pipetted into appropriate wells. Enzyme conjugate (100 μ L) was pipetted into each well, which were covered with adhesive strip, tapped gently on bench top to ensure thorough mixing and incubated for 1 hour at room temperature. Wells were washed three times with 300 μ L wash buffer, decanted and blotted dry on absorbent paper towels. TMB substrate (100 μ L) was pipetted into all wells, and incubated for 15 minutes at room temperature. Stop solution (50 μ L) was pipetted into each well, after which optical density was determined within 15 minutes at a wavelength of 450nm. Concentration of hs-CRP were obtained from standard curve (see appendix 9 for example standard curve), and obtained concentration was multiplied by the corresponding dilution factor. The intra and inter assay CV were 6.0% and 2.3% respectively.

3.2.2.5 Plasma TAG concentration determination

The quantitative enzymatic TAG determination kit measured glycerol, true and total TAG concentration in plasma (Sigma-Aldrich, Dorset, UK). Prior to the assay, free glycerol reagent (powder) was reconstituted by adding 40 mL of deionised water, the solution was mixed thoroughly by inversion and protected from light using foil. TAG reagent (powder) was reconstituted by adding 10 mL of deionised water. The solution was mixed thoroughly by inversion and protected from light using foil. Spectrophotometer wavelength was set at 540nm and water was used as reference.

Following reagent preparation, 800 µL of free glycerol reagent was pipetted into each cuvette. Water, standard, sample and control (10 µL each) were pipetted into labelled cuvettes respectively and incubated for 5 minutes at 37°C. Initial absorbance (IA) of blank, standard, sample and control were read at 540nm versus water as reference. After reading, 200 µL of reconstituted TAG reagent was pipetted into each cuvette, mixed thoroughly by inversion and incubated for a further 5 minutes. Final absorbance (FA) was read after last incubation at 540 nm versus water as reference.

Concentrations of total TAG were calculated using the formula:

$$\frac{(FA_{\text{sample}} - FA_{\text{blank}})}{(FA_{\text{standard}} - FA_{\text{blank}})} \times \text{concentration of standard}$$

Intra and inter assay CV for TAG were 2.6% and 6.2% respectively

3.2.2.6 Serum HDL-C concentration determination

Plasma HDL-C was measured using a quantitation kit (Sigma-Aldrich, Dorset, UK). Samples for HDL-C were first separated by adding equal volumes of precipitation buffer (100 µL) with sample (100 µL). The mixture was micro-centrifuged for 10 minutes at 2000 x g to ensure proper mixing, followed by incubation for 10 minutes. After incubation, tubes were further centrifuged for 10 minutes at room temperature, after this, the supernatant or HDL fraction was transferred to a new tube. Cholesterol standard solution (20 µL) was diluted with 140 µL cholesterol assay buffer to yield a 0.25 µg/µL standard from which serial dilutions were prepared. Cholesterol esterase and enzyme mix were reconstituted separately by adding 220 µL of cholesterol assay

buffer to each. The reaction mix contains 44 μL of cholesterol assay, 2 μL each of cholesterol probe, reconstituted cholesterol esterase and enzyme mix to yield a final volume of 50 μL .

Following sample and reagent preparation, sample (50 μL) and reaction mix (50 μL) were pipetted into each microplate well. This was thoroughly mixed using a horizontal plate shaker and incubated for 60 minutes at 37°C whilst protected from light. Optical density was read at 570nm. HDL-C concentrations were obtained from standard curve (see appendix 9 for example standard curve), obtained concentrations were multiplied by the corresponding dilution factor.

Intra and inter assay CV were 3.4% and 8.1% respectively.

3.2.2.7 Serum LDL-C concentration determination

LDL-C was determined using Friedewald formula (Friedewald et al., 1972).

Serum LDL-C = serum TC – serum HDL-C – (TRG/2.2)

3.2.2.8 Serum Non-High Density Lipoprotein-C (non-HDL-C) concentration determination

Non –HDL-C was determined using the formula: Non-HDL-C = TC - HDL-C.

3.2.2.9 Serum Total cholesterol (TC) concentration determination

The enzymatic-colorimetric cholesterol liquid (Alpha Laboratories, Eastleigh, UK) was used to determine TC concentration in plasma. Prior to the assay, 3 mL of deionised water was added to calibrator (lyophilised serum) to make a solution from which serial dilutions were produced. After this, 3 μL of standard, control and sample were pipetted into appropriate sterilised wells followed by the addition of reagent 1 (300 μL). Following this, microplate was incubated for 10 minutes, after incubation, optical density was obtained at a wavelength of 505nm (primary) and 700nm (reference). TC concentrations were obtained from the standard curve (see appendix 11 for example standard curve). Intra and inter assay CV were 5.2% and 6.4% respectively

3.2.2.10 Serum 8 - isoprostane concentration determination

A competitive *in vitro* immuno-enzymatic assay was used for the determination of 8-isoprostane concentration (Abcam, Cambridge, UK) in plasma. Prior to the assay, 10

x wash buffer was mixed with a stir bar whilst applying low gentle heat until a clear colorless solution was obtained. Following this, 25 mL wash buffer solution was diluted with 225 mL of deionised water to yield a final volume of 250 mL. One vial of horseradish peroxidase (HRP) conjugate (12 µL) was diluted with 12 mL of HRP buffer to prepare the 1x HRP conjugate. Sample dilution (25 mL) buffer was diluted with 225 mL deionised water to yield a final volume of 250 mL. The enclosed 2 µL standard vial filled with inert gas was first centrifuged, before 1.998 mL of 1 x sample dilution buffer was added to obtain a 2 mL stock solution. Several serial dilutions were made from the stock solution

Following reagent preparation, 200 µL of 1x sample dilution buffer was pipetted into blank wells, 100 µL of 1x sample dilution buffer was pipetted into maximum binding control wells. Standard, sample or control (100 µL each) was pipetted into the appropriate wells. HRP conjugate (100 µL) was pipetted into all wells except blank control wells, which wells were covered with adhesive strip and incubated for 2 hours. Following incubation, wells were washed manually three times with 400 µL wash buffer. After the final wash, the microplate was inverted and blotted dry on clean absorbent paper towels. After drying microplate, 200µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was pipetted into all wells, and incubated for 15-30 minutes at room temperature. Sulphuric acid (2N, 50 µL) was pipetted into all wells, mixed thoroughly before optical density was obtained within 30 minutes at a wavelength of 450nm (primary) and 570nm (reference). The concentration of 8-isoprostane was obtained from the standard curve (see appendix 9 for example standard curve). The intra and inter assay CV was 5.9% and 11.1% respectively.

3.2.2.11 Serum sE-selectin concentration determination

Plasma sE-selectin concentration (collected in heparin tubes) was determined using a quantitative sandwich enzyme immunoassay quantikine solid-phase ELISA kit (R&D Systems Europe, Abingdon, UK). The 96 well plates were pre-coated with a monoclonal antibody specific for naturally occurring human sE-selectin. Prior to assay, all samples were diluted 10-fold (30 µL of sample and 270 µL of calibrator diluent) to yield a final volume of 300 µL. Wash buffer (20 mL) concentrate was diluted by adding 480 mL of deionised water to yield a final volume of 500 mL. Substrate solutions (Colour A+B) were mixed together in equal volumes within 15

minutes of use and protected from light. The standard was reconstituted with 1.0 mL of deionised water and allowed to sit for at least 15 minutes before making serial dilutions.

Following sample and reagent preparation, assay diluent (100 μ L) was pipetted into each well. Standard, control, and sample (100 μ L each) were pipetted into appropriate wells, which were covered with adhesive strip and incubated for 2 hours. Following incubation, wells were decanted and washed four times manually with 400 mL wash buffer. After the final wash, the microplate was inverted and blotted dry against absorbent paper towels. Conjugate (200 μ L) was pipetted into each well and incubated for another 2 hours. After incubation, wells were washed four times and blotted dry against absorbent paper towels. Substrate solutions (colour A+B) (7 mL each) were mixed thoroughly, after which, 200 μ L each of the mix was pipetted into wells, the microplate was covered with foil paper to protect from light and left to incubate for 30 minutes. Following incubation, 50 μ L of stop solution was pipetted into each well and plate was tapped gently on bench top to ensure thorough mixing. The optical density of each well was determined within 30 minutes at 450nm (primary) and 570nm (reference) and the sE-selectin concentration was obtained from the standard curve (see appendix 8 for example standard curve). Obtained concentrations were multiplied by the corresponding dilution factor. Intra and inter assay coefficient of variation (CV) were 3.4% and 3.4% respectively.

3.2.2.12 Statistical analysis

Statistical analysis was performed using IBM SPSS statistics software (IBM SPSS version 22, NYC, USA). Statistical significance was set at $p < 0.05$). Continuous data were summarised as mean \pm standard deviation. The distributions of all continuous variables were examined for normality using the Shapiro Wilk statistics. For continuous variables that were normally distributed such as serum sE-selectin, non-HDL-C, hs-CRP, TAG, TC, LDL-C, and HDL-C, Pearsons correlation test was used to assess the association. For continuous variables that were not normally distributed including serum 25(OH)D, calcium, PTH and 8- isoprostanes, Spearman's correlation was used to assess the association.

3.3 RESULTS

3.3.1 Patient characteristics

The patient's physical characteristics are shown in **Table 3.3.1**. The proportion of males to female was significantly different, as the cohort had more males (62.5%) in comparison to females (37.5%).

Table. 3.3.1 Participants physical characteristics

Characteristics	Mean \pm SD
Age (years)	48.0 \pm 18.1
Male (62.5%); (n = 15)	47.1 \pm 18.8
Female (37.5%); (n = 9)	49.3 \pm 18.1

3.3.2. Baseline bone health and CVD biomarker concentrations

Serum sE-selectin, 8-Isoprostanes, TAG, TC, PTH, HDL-C, calcium, 25(OH)D concentrations, as well as LDL-C and non-HDL-C were not significantly different between males and females, however, serum hs-CRP concentration was different between males and females in the study cohort (**Table 3.3.2**). Serum mean (\pm SD) 25(OH)D concentration in both males (45.3 \pm 27.2, n = 15) and females (42.8 \pm 22.2, n = 9) fell under the vitamin D deficient category and was comparable to the corresponding sex and age matched general UK population of 42.4 \pm 21.0 nmol/L and 45.3 \pm 21.1 nmol/L respectively for men and women aged 19-64y and was 43.4 \pm 19 nmol/L and 47.9 \pm 21.8 nmol/L respectively for men and women aged above 65years (Public Health England, 2016).

Table 3.3.2. Baseline bone health and CVD biomarker concentrations

Biomarkers	Females Mean \pm SD	Males Mean \pm SD	Normal range	References
Serum 25(OH)D (nmol/L)	42.8 \pm 22.2	45.3 \pm 27.2	\geq 75 nmol/L	(Holick, 2007)
Serum PTH (pmol/L)	14.7 \pm 22.9	7.9 \pm 16.2	1.1-6.8 pmol/L	(Lombardi et al., 2008)
Serum calcium (mmol/L)	2.3 \pm 0.3	2.3 \pm 0.2	2.1-2.6 mmol/L	(Deng et al., 2014)
Serum hs-CRP (mg/L)	4.9 \pm 3.4	3.2 \pm 2.4	< 1.0 mg/L	(Buckley et al., 2009)
Serum 8-isoprostane (pg/mL)	25.2 \pm 24.3	32.0 \pm 18.5	35 \pm 6 pg/mL	(Milne et al., 2007)
Serum sE-selectin (ng/mL)	58.0 \pm 23.5	53.3 \pm 26.5		Not yet defined
Serum HDL-C (mmol/L)	0.6 \pm 0.1	0.5 \pm 0.1	1.0 -1.3 (men), 1.3-1.5 mmol/L (women)	(Glasziou et al., 2008)
LDL-C (mmol/L)	4.6 \pm 0.8	4.6 \pm 1.1	< 2.6 mmol/L	(Glasziou et al., 2008)
Serum TAG (mmol/L)	1.8 \pm 0.2	2.1 \pm 0.4	< 1.7 mmol/L	(Alberti et al., 2006)
Serum TC (mmol/L)	6.1 \pm 0.8	6.1 \pm 1.1	< 5.2 mmol/L	(Glasziou et al., 2008)
Serum non-HDL-C (mmol/L)	5.8 \pm 0.8	5.6 \pm 1.0	< 3.4 mmol/L	

3.3.3. Prevalence of vitamin D deficiency

Of the 24 calcium oxalate kidney stone patients recruited for this study, 3 (12.5%), 7 (29.2%) and 18 (75%) had serum 25(OH)D concentrations ≤ 25 nmol/L, 30nmol/L and 50nmol/L respectively (**Fig.3.3.1**). Of the cohort, 18 (75%) were vitamin D deficient and 4 (16.7%) were within the optimal limits (≥ 75 nmol/L) (Holick, 2007).

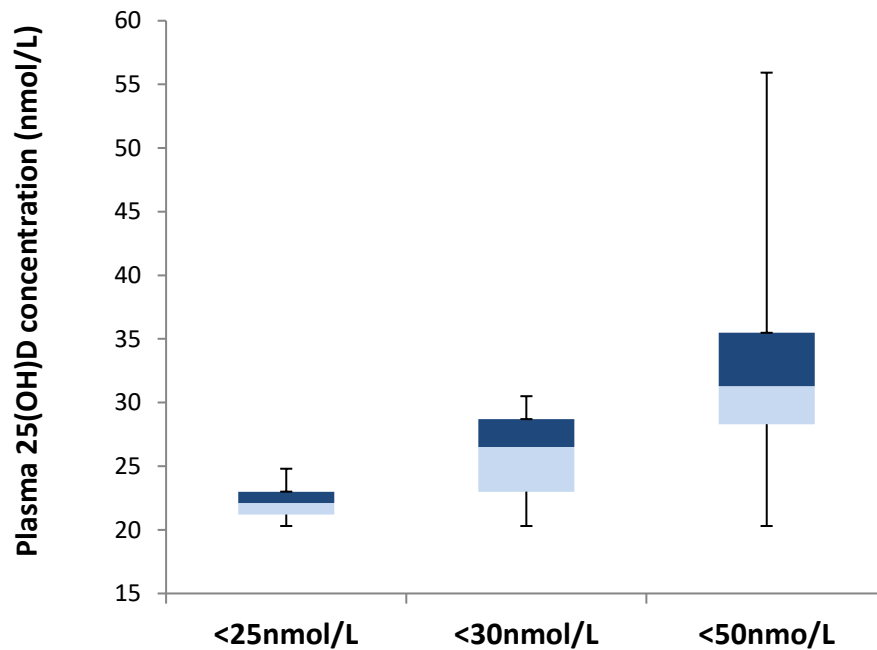


Fig 3.3.1 Box plot of plasma 25(OH)D concentrations showing prevalence of vitamin D deficiency/insufficiency

3.3.4. Association between serum 25(OH)D concentrations with bone and CVD biomarkers

No significant associations were observed between serum 25(OH)D concentration and the selected CVD/bone biomarkers. Similarly, no significant associations were also observed between serum 25(OH)D concentration and the selected CVD/bone biomarkers, when patient serum 25(OH)D concentrations were stratified into 25 (OH)D ≤ 30 nmol/L ($n = 7$) and 50 nmol/L ($n = 18$). Furthermore, when serum 25(OH)D concentration was split into percentiles according to 50% frequency distribution into ≥ 33 nmol/L and ≤ 33 nmol/L groups for both CVD/bone biomarkers no significant difference was observed between groups.

3.3.5. Association between bone and CVD biomarkers

Significant baseline positive associations were found between serum calcium and hs-CRP concentrations ($r = 0.58$, $P = 0.003$) (**Fig.3.3.2A**), serum calcium and sE-selectin concentrations ($r = 0.53$, $P = 0.007$) (**Fig.3.3.2B**), as well as a significant inverse association between sE-selectin and HDL-C ($r = -0.49$, $P = 0.013$) (**Fig.3.3.2C**). No significant associations were found amongst the remaining bone health and CVD biomarkers.

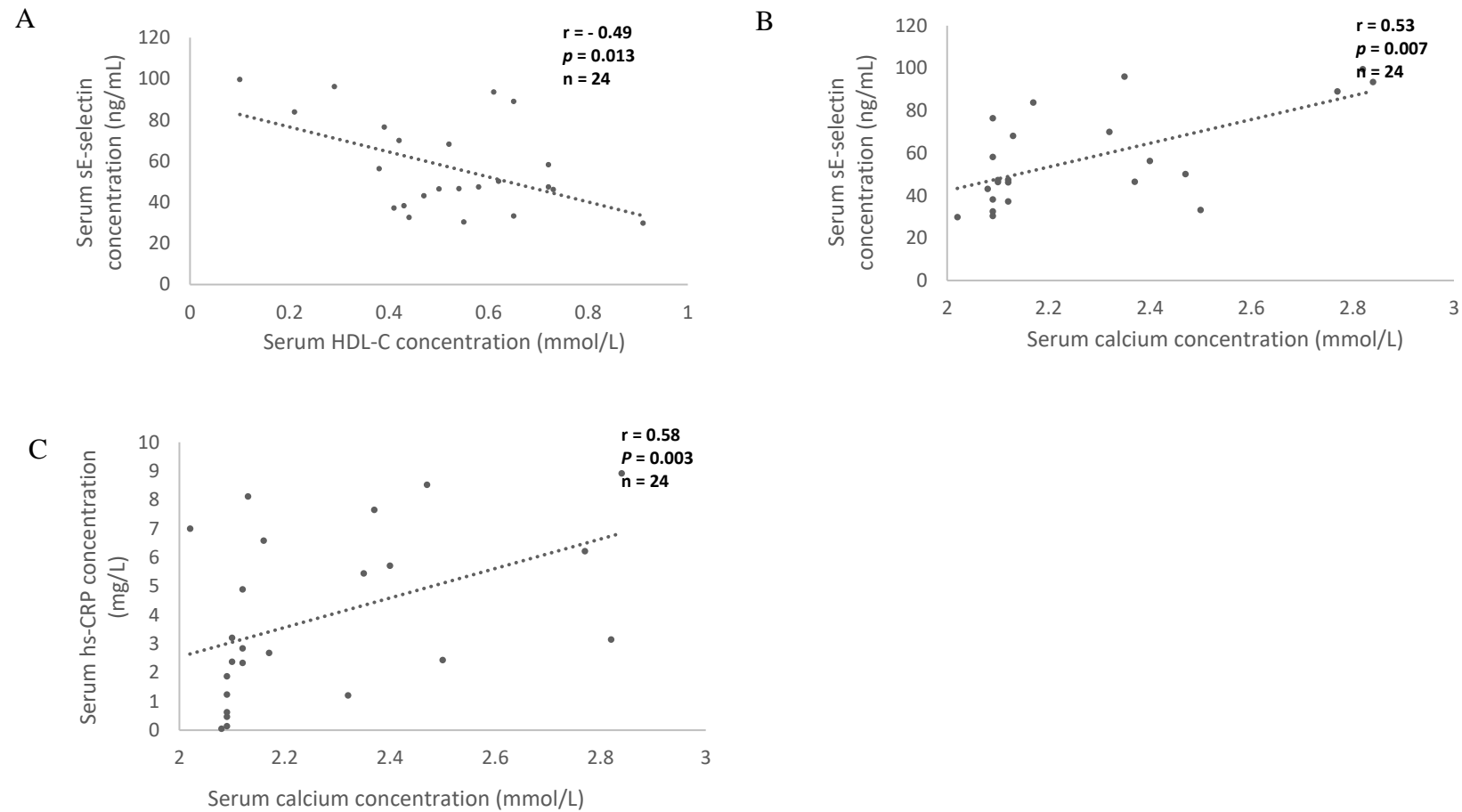


Fig.3.3.2. Scatter plot-showing associations between serum sE-selectin and (A) HDL-C concentration, (B) calcium concentration and (C) between serum hs-CRP and calcium concentration

3.4 DISCUSSION

To the best of my knowledge, this is the first cross-sectional study investigating in calcium oxalate kidney stone patients, the association between vitamin D status and selected CVD risk markers. The present cross-sectional study found a high prevalence of low serum 25(OH)D concentrations in the cohort as 3 (12.5%), 7 (29.2%) and 18 (75%) had serum 25(OH)D concentrations $\leq 25\text{nmol/L}$, $<30\text{nmol/L}$ and $<50\text{nmol/L}$ respectively. However, the mean ($\pm\text{SD}$) of 25(OH)D concentration are similar to those of the UK population.

No significant association was observed between serum 25(OH)D concentrations and the CVD markers measured. Interestingly, there was increased CVD risk in the study cohort, as all mean concentrations of bone health and CVD markers measured were above the normal ranges except for serum calcium and 8-isoprostanes concentration of which were within the normal range and serum HDL-C concentration which was below the normal range. Significant positive associations were observed between serum sE-selectin and calcium concentrations ($r = 0.53$, $P = 0.007$), serum hs-CRP and calcium concentrations ($r = 0.58$, $P = 0.003$). Furthermore, modest but significant inverse associations were observed between serum sE-selectin and HDL-C concentrations ($r = -0.49$, $P = 0.013$).

3.4.1 Vitamin D status

The present study in a cohort of calcium oxalate kidney stone patients demonstrated that low serum 25(OH)D concentrations below 50nmol/L mean ($\pm\text{SD}$) in males, $33.2 \pm 8.8 \text{ nmol/L}$ and females, $30.4 \pm 4.3 \text{ nmol/L}$ were highly prevalent in 75% of the cohort. However, no significant association was observed between the low serum 25(OH)D concentrations and the CVD biomarkers measured. The overall mean ($\pm\text{SD}$) serum 25(OH)D concentrations in the present study for males ($45.3 \pm 27.2 \text{ nmol/L}$) and females ($42.8 \pm 22.2\text{nmol/L}$) were comparable to the corresponding sex and age matched UK population. The mean ($\pm\text{SD}$) serum 25(OH)D concentration for the UK adult population was $42.4 \pm 21.0 \text{ nmol/L}$ and $45.3 \pm 21.1 \text{ nmol/L}$ respectively for men and women aged 19-64 years and was $43.4 \pm 19 \text{ nmol/L}$ and $47.9 \pm 21.8 \text{ nmol/L}$ respectively for men and women aged above 65years (Public Health England, 2016). Among adults in the general UK population, 22% men and

15% women aged 19-64 years and 21% men and 9% women above 65 years had serum 25(OH)D concentrations below 25nmol/L (Public Health England, 2016).

These findings complement recent observations suggesting that vitamin D insufficiency is strongly associated with kidney stone incidence among adult participants. Among 884 idiopathic calcium kidney stones patients and 967 controls median (range) serum 25(OH)D <50 nmol/L was lower in the kidney stones patients compared with controls 45 (30-60) vs. 57.5 (35-70) nmol/L, ($P = 0.02$) (Ticinesi et al., 2016). Similarly, another case control study found mean (\pm SD) serum 25(OH)D concentrations <50 nmol/L in patients with calcium kidney stone compared to healthy controls, 64.3 ± 25.5 vs. 71 ± 23.7 nmol/L, ($P = 0.02$), and only 28% of the kidney stone patients and 15.7% of controls had vitamin D insufficiency (Giron-Prieto et al., 2016). Compared to these studies which were performed in Italy and Spain respectively, the present study cohort had much lower mean serum 25(OH)D concentrations and this may be attributed to the UK being a less temperate or sunny region compared to Italy and Spain, resulting in the reduced production of vitamin D.

Several potential mechanisms may be responsible for the low serum 25(OH)D concentrations observed in the present study. The reduced renal CYP27B1 activity observed in kidney disease is one such mechanism (Vanholder et al., 1993). Observations from a study in patients with mild to moderate kidney failure suggest that uric acid, a uraemic biologic fluid, suppresses vitamin D metabolism by reducing renal CYP27B1 activity which leads to reduced vitamin D synthesis (Vanholder et al., 1993). Secondly, increased circulating FGF-23 concentrations observed in kidney dysfunction promotes the transcription of CYP24A1, causing increased 25(OH)D and 1,25(OH)₂D catabolism resulting in low serum 25(OH)D concentrations (Jones et al., 2012; Petkovich & Jones, 2011). Findings from rat models and renal biopsy tissue from patients with kidney disease have shown a high expression of circulating CYP24A1 mRNA in diseased compared to healthy renal tissue (Helvig et al., 2010). The low serum 25(OH)D concentration observed in the present study cohort could also be ascribed to the albuminuria caused by impaired kidney function in stone formation (Rule, Krambeck, & Lieske, 2011). It has been suggested that albuminuria may result in high urinary loss of VDBP, leading to increased renal loss of all vitamin D metabolites (Andress, 2005). It was reported that

in patients with nephrotic albuminuria, there were remarkably low serum concentrations of serum 25(OH)D ($< 75\text{nmol/L}$) (Gonzalez et al., 2004).

3.4.2. Associations between vitamin D status and CVD biomarkers

No significant associations were observed between serum 25(OH)D concentrations and the selected CVD risk biomarkers measured in calcium oxalate kidney stone patients. Studies which have investigated the association between serum 25(OH)D concentrations and CVD risk biomarkers in calcium oxalate kidney stone patients are lacking. However, low vitamin D concentrations have been reported to be associated with increased CVD risk in CKD patients. A cross-sectional study in 101 stage 4-5 non-dialysis, CKD patients and 40 healthy controls found that serum hs-CRP concentration was inversely associated with low concentrations of serum 25(OH)D $< 50\text{nmol/L}$ ($r = -0.2$, $P = 0.047$) in the CKD patients compared to controls with normal kidney function (Yadav et al., 2012). The inverse association found in this study may be attributed to the fact that stage 4-5 CKD patients are more likely to have severe vitamin D deficiency compared with calcium oxalate kidney stone patients (Jabbar et al., 2009). This is because there are more significant reductions in vitamin D metabolism as a result of more severe proteinuria in stage 4-5 CKD (Cuppari, Carvalho, & Draibe, 2008) compared to kidney stone disease (Rule et al., 2009). The small sample size in the present study pilot may be another reason why an association was not observed between serum 25(OH)D concentrations and CVD risk biomarkers.

3.4.3. Increased CVD risk in the study cohort

In the present study, there was increased CVD risk, as mean concentrations of all biomarkers were above the normal range except for a normal range serum calcium and 8-Isoprostanes concentration, however, serum HDL-C concentration that was below the normal range. Consistent with the present study findings of a normal serum mean \pm (SD) calcium concentration in males $2.3 \pm 0.2\text{ mmol/L}$ and in females $2.3 \pm 0.3\text{ mmol/L}$, a study performed in 236 kidney stone patients also found normal serum mean \pm (SD) calcium concentrations (Pipili & Oreopoulos, 2013). Similarly, no significant difference was found between serum mean (\pm SD) calcium concentrations of kidney stone disease patients compared to those without kidney stone disease 8.87 ± 0.65 vs. $8.65 \pm 0.60\text{ mg/dL}$, $P = 0.180$) (Hasna et al., 2015).

These observations suggest that despite the disturbance in calcium homeostasis observed with kidney dysfunction, serum and intracellular calcium concentrations are tightly regulated, as several vital metabolic processes are unable to tolerate a significant deviation from the normal range (Moe, 2008). Serum mean (\pm SD) PTH concentrations were observed to be high in males 14.7 ± 22.9 pmol/L and females 7.9 ± 16.2 pmol/L in the present study. Similar to the present study, a study in 236 recurrent kidney stone disease patients also reported increased serum PTH concentrations (> 7.5 pmol/L) in a quarter of the cohort (Pipili & Oreopoulos, 2013).

Oxidative stress, determined by serum 8-Isoprostanes concentration in the present study were within the normal range mean (\pm SD) in males, 32.0 ± 18.5 pg/mL and in females 25.2 ± 32.0 pg/mL. This observation in the present study was unexpected as calcium oxalate kidney stone patients have been reported to excrete increased amount of α -glutathione S-transferase (α -GST), MDA and Thiobarbituric acid reactive substances (TBARS) in their urine, demonstrating increased oxidative stress (Huang, Ma, Chen, & Chen, 2003). Additionally, in comparison to people with normal kidney function, kidney stone patients have been shown to have high oxidative stress markers such as higher plasma and urinary MDA and reduced anti-oxidants such as glutathione (GSH), cellular glutathione peroxidase (cGPx) activity, protein thiol and vitamin E (Tungsanga, Sriboonlue, Futrakul, Yachantha, & Tosukhowong, 2005). Furthermore, serum 8-hydroxydeoxyguanosine concentration, a marker of oxidative damage of the DNA has been reported to be increased in the urine of kidney stone patients compared with controls (Boonla, Wunsuwan, Tungsanga, & Tosukhowong, 2007).

Kidney stone disease is characterised by low-grade inflammation (Shoag & Eisner, 2014), and in the present study, inflammation measured by serum mean \pm (SD) hs-CRP concentrations were above the normal range in males, 3.2 ± 2.4 mg/L and females, 4.9 ± 3.4 mg/L. Consistent with this observation, a significant increase in serum mean \pm (SD) hs-CRP concentration has also been reported in T2DM patients with kidney stone disease compared with T2DM patients without kidney stone disease 115.3 ± 50.9 vs. 79.4 ± 54.6 μ g/L (Hasna et al., 2015). Additionally, significantly higher mRNA expression of IL-6 and MCP-1 (inflammatory biomarkers) have been reported in 29 renal biopsy tissue collected from kidneys containing stones compared to the 30 renal biopsy tissue of healthy participants

(Boonla et al., 2008). These observations indicate that the impaired renal function observed in kidney stone disease is associated with increased inflammatory biomarkers.

Exposure of the proximal tubule epithelial cells to calcium oxalate has been shown to be associated with renal cell injury and inflammation (Evan et al., 2010), as calcium oxalate deposition was found to attract several inflammatory cells including leukocytes, monocytes, and macrophages in male Sprague-Dawley rats (Khan, Glenton, & Byer, 2006). Additionally, in rat kidney cell culture, the exposure of the renal epithelium to calcium oxalate crystals caused the increased secretion of MCP-1 suggesting calcium oxalate kidney stone patients would have increased inflammation (Umekawa, Chegini, & Khan, 2002; Umekawa et al., 2003).

In the present study, mean (\pm SD) serum concentrations of soluble E-selectin were increased in both males 53.3 ± 26.5 ng/mL and females 58.0 ± 23.5 ng/mL. Although a normal range of serum 8-isoprostanes concentration was reported, endothelial dysfunction in calcium oxalate kidney stone patients is in part mediated by oxidative stress (Khan, 2014; Weiss et al., 2000). One of the mechanisms by which oxidative stress lead to endothelial dysfunction is by disrupting the NO signalling pathway, as a result, NO losses its anti-inflammatory, vasodilatory, as well as its vasoprotective properties resulting in endothelial dysfunction (Förstermann, 2010). Additionally, on exposure of the renal epithelium to ROS, NO is converted to peroxynitrite, a powerful oxidant that causes the oxidation of anti-oxidants such as cysteine tetrahydrobiopterin and glutathione (Szabó, Ischiropoulos, & Radi, 2007). Peroxynitrite, causes reduced production of NO by inducing the oxidation of the zinc thiolate centre of NO synthase (Joshi et al., 2013), and a decrease in NO production will subsequently lead to endothelial dysfunction.

3.4.4. Increased risk of dyslipidaemia in calcium oxalate kidney stone patients

In the present study, serum mean (\pm SD) TC concentrations, in males and females, 6.1 ± 1.1 and 6.1 ± 0.8 mmol/L, serum mean (\pm SD) TAG concentrations in males and females 1.8 ± 0.2 mmol/L and 2.1 ± 0.4 mmol/L were slightly above the normal range. Additionally, mean (\pm SD) LDL-C concentrations in males and females 4.6 ± 1.1 and 4.6 ± 0.8 mmol/L and mean (\pm SD) non-HDL-C concentration in males and in females 5.8 ± 0.8 and 5.6 ± 0.8 mmol/L were also above the normal range. However,

serum mean (\pm SD) HDL-C concentration was decreased in both males and females: 0.5 ± 0.1 and 0.6 ± 0.1 mmol/L respectively. Consistent with the present study findings, a cross-sectional study of 60 T2DM patients with and without kidney stone demonstrated significantly increased serum mean (\pm SD) TC concentration, 163.6 ± 46.7 vs. 137.2 ± 28.0 mg/dL, ($P = 0.010$). This study showed increased) serum mean (\pm SD) TAG concentration, 143.5 ± 61.1 vs. 91.7 ± 24.5 mg/dL, ($P < 0.001$), mean (\pm SD) LDL-C concentration 105.6 ± 44.4 vs. 81.7 ± 24.6 mg/dL, ($P = 0.011$). The cross-sectional study also showed decreased serum mean (\pm SD) HDL-C concentration 29.1 ± 5.4 vs. 37.8 ± 12.5 mg/dL ($P = 0.0012$) in the T2DM patients with kidney stones compared to T2DM patients without kidney stones (Hasna et al., 2015).

Similarly, in 13 patients with recurrent kidney stones, a significant increase in serum TC, TAG, LDL-C and decrease in HDL-C concentration was reported in the kidney stone patients compared with the healthy controls (Abate, Chandalia, Cabo-Chan, Moe, & Sakhaee, 2004). Furthermore, a study in 49 kidney stone patients and controls demonstrated that serum TC and TAG concentration were increased in the kidney stone patients (Inci, Demirtas, Sarli, Akinsal, & Baydilli, 2012). These observations in addition to those of the present study suggest kidney stone disease is associated with altered lipid profile. It has also been suggested that inflammation may lead to an increase in circulating serum lipid concentrations by reducing peripheral lipoprotein lipase activity (Castrillo et al., 2003). Cytokines may also stimulate the liver to increase the secretion of very-low density lipoproteins (VLDL), resulting in dyslipidaemia (Esteve, Ricart, & Fernandez-Real, 2005).

Kidney disease is associated with low serum HDL-C concentration, as observed in the present study. A study in 2442 kidney stone patients also reported a low serum HDL-C concentration which was associated with increased oxalate (Torricelli et al., 2014). The beneficial properties of HDL-C have been reported to be altered even in early CKD and show a graded association with eGFR (Shroff et al., 2014). More emphasis is laid on serum HDL-C concentrations as HDL-C exerts cardio-protective function in the vasculature by preventing the development of endothelial dysfunction and through the suppression of LDL-mediated oxidative damage to the arterial wall (Kim, Montagnani, Chandrasekran, & Quon, 2012). HDL-C also exerts anti-inflammatory action on the vasculature by suppressing the expression of E-selectin

induced by interleukins (Barter et al., 2004; Cockerill et al., 2001), through the inhibition of sphingosine kinase, an enzyme involved in the modulation of endothelial adhesion molecule by TNF- α (Argraves & Argraves, 2007; Sattler & Levkau, 2009). Thus, a low serum HDL-C concentration indicates increased CVD risk in the present study cohort.

3.4.5. Associations between CVD biomarkers

There was a significant positive association between serum sE-selectin and calcium concentration ($r = 0.53$, $P = 0.007$) in the present study. No association between serum sE-selectin and calcium concentration has been reported in calcium oxalate kidney stones patients to the best of my knowledge, this may be attributed to the fact that serum calcium concentrations are tightly regulated (Moe, 2008). Additionally, the blood is not a major store of body calcium, 99.9% of body calcium is stored in the bone and could also be deposited in the intima and media of arteries in people with renal disease (Moe & Chen, 2008). Furthermore, the constant exposure of VSMCs to increased concentrations of calcium and phosphate directly induce the osteogenic transformation of VSMCs, initiating and promoting vascular calcification (Shanahan et al., 2011).

There is evidence that impaired calcium homeostasis in kidney disease patients results in arterial calcification particularly coronary artery calcification that reflects intense atherosclerosis (Amann, 2008; Nakamura et al., 2009). The pathological pathways responsible for kidney stone formation are also implicated in coronary artery calcification (CAC) that leads to atherosclerosis (Yiu et al., 2015). A cross sectional study in 984 people without CVD or diabetes found coronary artery calcium (CAC), a marker of coronary artery damage in atherosclerosis to be associated with increased serum E-selectin concentrations (de Almeida-Pititto et al., 2016). This observation supports the present study finding of a positive association between serum sE-selectin and calcium concentration.

The present study also found significant positive associations between serum hs-CRP and calcium concentration ($r = 0.58$, $P = 0.003$), suggesting inflammation plays a significant role in calcium deposition in arteries, as vascular calcification has been shown to be induced by inflammatory cytokines such as TNF- α and CRP (Moe & Chen, 2005). CAC was not assessed in the present study, as this study was a

retrospective cross-sectional pilot, however, measuring CAC may have explained the association better, as a study in kidney stones patients reported increased risk of vascular calcification (Kleinman et al., 2004). A study of 14,584 Korean normotensive, pre-hypertensive and hypertensive subjects found increasing serum hs-CRP concentration ($>2\text{mg/L}$) following increasing CAC score > 0 in normal and pre-hypertensive subjects. High serum hs-CRP concentration was significantly associated with CAC score in the normotensive group (OR 1.55, 95% CI 1.11–2.16, $P = 0.010$) but not in the prehypertensive group (Sung et al., 2014).

Furthermore, modest but significant inverse associations were observed between serum sE-selectin and HDL-C concentrations ($r = -0.49$, $P = 0.013$), which indicates an increased risk of endothelial dysfunction and atherosclerosis with low serum/plasma HDL concentrations. Increased sE-selectin concentration may possibly be the mechanism by which reduced serum/plasma HDL-C concentrations promotes atherogenesis and causes severe athero-thrombotic events (Calabresi et al., 2002). One study, incubated human aortic endothelial cells along with HDL isolated from CKD and healthy children to measure the endothelial properties of HDL. This study demonstrated that the healthy HDL-C increased endothelial NO production, whilst CKD HDL-C markedly repressed endothelial NO production and increased endothelial VCAM-1 expression (Shroff et al., 2014). Consistent with this observation, low serum HDL-C concentration was found to be inversely associated with serum sE-selectin concentration in individuals with low serum HDL-C (Calabresi et al., 2002). Additionally, it has also been reported that the serum concentrations of sE-selectin are high in people with low HDL-C concentration (Soro-Paavonen, Westerbacka, Ehnholm, & Taskinen, 2006).

A study aimed at determining whether the modification of HDL-C in CKD patients leads to alterations of its endothelial effects by modulation of the innate immune system, observed that CKD HDL-C lost its endothelial-protective properties and became a toxic particle that induced endothelial dysfunction compared to healthy HDL-C (Speer et al., 2013). It was also observed that symmetric-dimethylarginine (SDMA), a uraemic toxin in the HDL-C fraction was responsible for the detrimental vascular action of this toxic HDL-C. Additionally, this study demonstrated that the altered HDL-C induced an endothelial TLR-2 response via a TLR-1- and TLR-6-

coreceptor and NF- κ B-independent pathway resulting in increased endothelial superoxide production and reduced NO bioavailability (Speer et al., 2013).

All of the patients in this cohort were diagnosed with calcium oxalate stones, which injure cells and obstruct cellular functions by damaging cell membranes, producing lipid mediators such as prostaglandins and releasing of ROS leading to oxidative stress. Although little is known about the association between serum 25(OH)D concentration and kidney stone disease, it may not be correct to attribute the high prevalence of low vitamin D status observed in the present study to the presence of kidney stones. This is because the mean serum 25(OH)D concentration of the cohort was comparable to that of the general UK population. Findings from a large US population based cross-sectional study in 16286 adults with previous kidney stones and non-kidney stone formers did not demonstrate a significant difference in serum 25(OH)D concentrations amongst kidney stone patients and healthy controls (Tang et al., 2012).

3.4.6. Study limitations

As this study is a retrospective analysis of stored serum samples collected in a cross-section of calcium oxalate kidney stone disease patients, sample size was small, and the bone health/CVD parameters were only evaluated at one-time point (baseline/pre-operation). Secondly, aged matched controls to make comparisons were lacking, as serum samples were collected retrospectively. Renal function and circulating FGF23 concentrations were not determined. Measuring eGFR and circulating FGF23 concentrations in the present study cohort may explain better the reductions observed in serum 25(OH)D concentrations. Finally, due to budget constraints, only some surrogate biomarkers of CVD were studied, a more comprehensive assessment of cardiovascular outcomes may demonstrate some associations.

3.4.7. Future perspectives

Further research using a larger population with age-matched controls is required. Research investigating indices of atherosclerosis such as CAC would likely provide distinct information regarding CVD risk in calcium oxalate kidney stones patients. Further studies are also required to investigate whether calcium oxalate kidney stone

formers have reduced concentrations of urinary osteopontin, as a deficiency leads to vascular calcification, and to date only one study in humans is available (Bautista, Denstedt, Chambers, & Harris, 1996). Vitamin D supplementation in this population is required, as this may not only decrease PTH secretion, it would also improve VDR expression and activities that would in turn exert reno-protective effects by downregulating RAS activity. Additionally, assessing renal function and circulating FGF-23 concentration in a similar population is required. Studies evaluating the effect of vitamin D supplementation in order to improve deficient vitamin D levels to the optimal serum 25(OH)D concentration and the dose of vitamin D required for preventing CVD in this cohort needs further research. Given its reported safety together with its beneficial reno-protective effects, there is optimism that correcting vitamin D deficiency may lead to better outcomes for patients with calcium oxalate kidney stone disease.

3.4.8. Conclusion

Overall, this cross-sectional study demonstrated that in calcium oxalate kidney stones patients, the prevalence of vitamin D deficiency determined by serum 25(OH)D concentration was high. However, the mean (\pm SD) of 25(OH)D concentration are similar to those of the UK population. No significant association was found between the low serum 25(OH)D concentrations and the CVD biomarkers measured. There was increased CVD risk in the study cohort and significant associations were observed between sE-selectin with calcium, hs-CRP with calcium and with sE-selectin with HDL-C.

4.0 GENERAL DISCUSSION AND CONCLUSION

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Vitamin D deficiency (serum 25(OH)D concentrations < 25nmol/L) is highly prevalent in the UK. It affects a fifth of adults aged 19 years and above and a sixth of children aged 11 to 18 years (Bates et al., 2014). CVD is responsible for 26% of all deaths in UK whilst, diabetes has been reported to affect 4.5 million people in the UK, 90% of which suffer from T2DM (Public Health England, 2015). Vitamin D deficiency has been implicated in the pathomechanism of different diseases including diabetes mellitus, cancer, kidney disease and CVD. Potential consequences of vitamin D deficiency include bone disease, inflammation, increased insulin resistance and oxidative stress, endothelial dysfunction, hypertension and arterial stiffness (Menezes et al., 2014). Vitamin D deficiency has also been greatly associated with increasing adiposity (Vimaleswaran et al., 2013), possibly the result of enhanced adipocyte sequestering, as well as suppressed 25(OH)D synthesis by adipocytokines secreted by adipose tissue (Ding et al., 2010). The kidneys are the primary target organs affected by calcium regulatory hormones such as vitamin D, and a decline in kidney function has been reported to reduce vitamin D synthesis (Bosworth & de Boer, 2013). The reduction of vitamin D synthesis in kidney disease have been attributed to increased production of FGF-23, which downregulates the expression of renal 1 α -hydroxylase (CYP27B1) and upregulates 24-hydroxylase (CYP24A1) activity (Hasegawa et al., 2010).

Given the high prevalence of vitamin D deficiency and cardiometabolic disease in the general UK population, particularly in overweight/obese people and the inverse associations between low vitamin D and cardiometabolic disease, improving vitamin D status by supplementation has been proposed as a favourable strategy for cardiometabolic disease prevention (Wang, Song, et al., 2012), as few foods naturally contain vitamin D (Lu et al., 2007). It has therefore become increasingly important to investigate the effect of vitamin D₃ therapy in cohorts reported to be at increased risk of both vitamin D deficiency and cardiometabolic disease as most available RCT trials have tested low daily doses or large single doses. Similarly, it is also important to assess participant's dietary status in order to determine the possible influence of dietary supplementation.

The present PhD research comprises of 2 studies aimed at investigating the effect and association of vitamin D with metabolic and CVD markers. The primary purpose of the first study was to investigate whether the administration of 5,000IU (125µg) vitamin D₃ for 8 weeks would significantly improve vitamin D status and the selected cardiometabolic markers measured in overweight and obese adult males. Additionally, a further hypothesis was to investigate whether improved vitamin D status would be significantly associated with the selected cardiometabolic markers measured. The administration of 5,000IU (125µg) vitamin D₃ for 8 weeks increased mean (\pm SD) plasma 25(OH)D concentrations significantly from a baseline concentration of 38.4 ± 15.9 to 64.2 ± 19.5 nmol/L ($P < 0.001$) at 4 weeks and 72.8 ± 16.1 nmol/L, ($P < 0.001$) post intervention. All study participants recruited for this study had a baseline plasma 25(OH)D concentration < 75 nmol/L, of these, vitamin D deficiency, defined as plasma 25(OH)D concentrations < 25 nmol/L by SACN, 2015, was present in 22.2% (12/54) of participants. Vitamin D deficiency, defined as plasma 25(OH)D concentrations < 30 nmol/L by IOM, 2011 was present in 35.2% (19/54) and plasma 25(OH)D concentration < 50 nmol/L concentrations was present in 64.8% (35/54) of the cohort at baseline.

Following 8 weeks vitamin D supplementation, 45.8% (11/24) of participants in the vitamin D group achieved plasma 25(OH)D concentrations > 75 nmol/L post intervention, whilst the remaining 54.2% (13/24) did not achieve plasma 25(OH)D concentrations > 75 nmol/L despite the high daily dose administered. This observation suggest 54.2% (13/24) of cohort in the vitamin D group did not achieve the optimal plasma 25(OH)D concentrations required for non-skeletal benefits (Bischoff-Ferrari, 2008). This finding also supports the hypothesis that vitamin D metabolism is diminished with increasing BMI (Vimalleswaran et al., 2013). The lipid component of a meal has been suggested to improve vitamin D absorption (Mulligan & Licata, 2010), and the participants mean dietary fat intake were within those of the DRV and age matched UK male population. This indicates that participants consumed enough fat to improve vitamin D absorption, thus, the reduced plasma 25(OH)D concentration would be ascribed to other unknown factors and not inadequate fat consumption.

The inability of a greater percentage of study participants to reach the 75nmol/L threshold could also be ascribed to adipose sequestration, as it has been reported in humans that about 17% of orally-administered vitamin D was stored in adipose tissue (Heaney et al., 2010). Baseline vitamin D status may be another explanation, as only 22.2% (12/54) participants had plasma 25(OH)D concentrations <25nmol/L at baseline, and intestinal absorption of vitamin D may be higher in vitamin D deficient individuals, suggesting that the benefits or risks of vitamin D supplementation may depend on initial levels of 25(OH)D (Apukhovskaia et al., 1990; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad, & Weaver, 2011).

The reduced expression of both 25-hydroxylase CYP2J and 1- α hydroxylase CYP27B1 in adiposity could be another potential explanation for the reduced ability of some participants in the vitamin D group to achieve plasma 25(OH)D concentrations above 75nmol/L. (Wamberg, Christiansen, et al., 2013). Circulating VDBP may be another potential explanation for the reduced plasma 25(OH)D, as circulating VDBP, which has a greater binding affinity for 25(OH)D compared to 1,25(OH)₂D (Gozdzik et al., 2011; Hart et al., 2005) is decreased in acute inflammatory conditions as is observed with increasing BMI (Waldron et al., 2013; Yousefzadeh et al., 2014).

Furthermore, VDR genotype polymorphisms is reported to be associated with adiposity (Gu et al., 2009; Ochs-Balcom et al., 2011). VDR genotype polymorphism particularly VDR *ff* genotype have been reported to have decreased response to vitamin D intake (Neyestani et al., 2013). Thus, VDR genotype polymorphism in adiposity may be an additional explanation for the reduced individual response to the high dose orally administered vitamin D in the present study.

The change in 25(OH)D concentration was inversely associated with change in plasma glucose concentrations ($r = -0.408$, $P = 0.048$), as well as change in HDL-C concentrations ($r = -0.503$, $P = 0.012$) and positively associated with change in aortic central pulse pressure ($r = 0.437$, $P = 0.033$) at week 4. Improved 25(OH)D concentration post intervention was inversely associated with change in brachial augmentation index ($r = 0.446$, $P = 0.029$). These observations suggest a link between decreasing/or increasing plasma 25(OH)D concentration with increasing/improved insulin resistance, dyslipidaemia and arterial stiffness.

No significant effect of vitamin D supplementation was observed in the cardiometabolic markers including endothelial function, arterial stiffness, blood pressure and RAS, lipid profile, oxidative stress, and inflammation and glucose homeostasis parameters between treatment groups. However, vitamin D supplementation significantly reduced mean (\pm SD) brachial pulse pressure from baseline (52.3 ± 9.2 vs. 53.7 ± 9.2 mmHg to 49.4 ± 3.9 vs. 53.5 ± 6.4 mmHg ($P = 0.011$) at 4 weeks and to 50.6 ± 5.8 vs. 55.8 ± 8.7 mmHg ($P = 0.027$) post intervention in the vitamin D but not in the placebo group. Additionally, despite improvements in vitamin D status, BMI, body weight and waist circumference remained unchanged by vitamin D therapy, indicating vitamin D does not reduce body weight.

Potential explanations for the lack of beneficial effects of vitamin D supplementation include the following. Firstly, the optimal serum/plasma 25(OH)D concentrations required to prevent CVD risk has not been established, as a threshold effect which varies with different baseline serum/plasma 25(OH)D concentrations has been reported (Heaney, 2012; Mozos & Marginean, 2015). The wide range of baseline plasma 25(OH)D concentration among participants could also potentially produce heterogeneous biological effects.

Secondly, the use of a non-hypertensive and non-diabetic cohort at baseline may be the possible reason the present study did not find significant changes in SBP, DBP and MAP compared to studies which did (von Hurst et al., 2010; Witham et al., 2009). Thirdly, the use of young cohort below 50 years could be another reason, as a majority would have had fewer anatomical changes to their arterial tree, since major changes to the arterial tree occurs between ages 50-60 (Reshetnik et al., 2016). Additionally, the lack of an effect of vitamin D supplementation on BMI, body weight and waist circumference may be attributed to using BMI to assess body fat, as BMI does not measure body fat distribution.

The assessment of vitamin D is further complicated by the fact that CVD is a heterogeneous category, and it is possible that vitamin D has a different effect in a specific cardiometabolic outcome (Rosen et al., 2012). The estimated mean (\pm SD) vitamin D intake (2.5 ± 2.6 μ g/d) was insufficient compared to the recommended RNI of 10 μ g/d, suggesting that vitamin D supplementation would be required to

raise the habitual intake in the UK to 10µg/d, particularly in the overweight and obese.

The second study in the present project was aimed at investigating the prevalence of vitamin D deficiency, and its association with selected CVD risk markers in a cross-section of calcium oxalate kidney stone patients. This study demonstrated that out of the 24 calcium oxalate kidney stone patients recruited for this study, 3 (12.5%), 7 (29.2%) and 18 (75%) had serum 25(OH)D concentrations $\leq 25\text{nmol/L}$, $\leq 30\text{nmol/L}$ and $\leq 50\text{nmol/L}$ respectively. Of the cohort 3 (12.5%), 7 (29.2%) and 18 (75%) were vitamin D deficient respectively according to definitions by the Scientific Advisory Committee on Nutrition (SACN, 2016), Institute of Medicine (IOM, 2011) Holick, (2007). However, 2 (8.3%) were insufficient and only 4 (16.7%) were within the optimal limits ($\geq 75\text{ nmol/L}$) (Holick, 2007). These observations suggest a high prevalence of low serum 25(OH)D concentrations in this cohort.

Potential mechanisms responsible for the low serum 25(OH)D concentrations observed in the study cohort include increased circulating FGF-23 concentrations (Jones et al., 2012). Increased circulating FGF-23 concentrations in renal disease has been reported to stimulate the transcription of CYP24A1, resulting in increased 25(OH)D and 1,25(OH)₂D catabolism and low serum 25(OH)D concentrations (Jones et al., 2012). Albuminuria caused by impaired kidney function in stone formation could be another explanation, as albuminuria leads to high urinary loss of VDBP, leading to increased renal loss of all vitamin D metabolites (Andress, 2005).

No significant associations were observed between serum 25(OH)D concentrations and the selected CVD risk biomarkers measured in calcium oxalate kidney stone patients as hypothesised. This observation may suggest that clinical disease decreases serum vitamin D concentration causing insufficiency but does not cause ill health (Theodoratou et al., 2014). Interestingly, there was increased CVD risk in the study cohort, as all biomarkers except for serum calcium and 8-Isoprostanes concentration, which was within the normal range and serum HDL-C concentration that was below the normal reference range, all the other CVD biomarkers measured were above the normal reference range. These observations, particularly the increased serum inflammatory and endothelial function, TC, TAG, non-HDL-C and LDL-C as well as

reduced serum HDL-C markers are similar to findings of other studies in kidney stones patients (Evan et al., 2010; Hasna et al., 2015; Khan, 2014).

Significant positive associations were observed between serum sE-selectin and calcium concentrations ($r = 0.53$, $P = 0.007$), suggesting the disturbance observed with declining kidney function may result in the deposition of calcium in the arteries resulting in coronary artery calcification that leads to atherosclerosis (Yiu et al., 2015). Positive association between serum hs-CRP and calcium concentrations ($r = 0.58$, $P = 0.003$) were observed, indicating a role for inflammation in vascular calcification (Moe & Chen, 2005). Coronary artery calcification or calcium levels were not assessed in the present study, which may have explained the association better, as a study in humans have reported kidney stones patients to be at increased risk of vascular calcification (Kleinman et al., 2004).

Furthermore, inverse associations were observed between serum sE-selectin and HDL-C concentrations ($r = -0.49$, $P = 0.013$), indicating an increased risk of endothelial dysfunction with low serum HDL concentrations. Similar to this finding, human aortic endothelial cells incubated with HDL isolated from children with CKD, demonstrated that CKD HDL-C markedly repressed endothelial NO production and increased endothelial VCAM-1 expression (Shroff et al., 2014).

Although little is known about the association between serum 25(OH)D concentrations and calcium oxalate kidney stone disease, it may not be appropriate to attribute the high prevalence of vitamin D deficiency and insufficiency observed in this cohort to the presence of kidney stone disease, as vitamin D deficiency is highly prevalent in the general UK population.

Additionally, in the cross-sectional study, the low serum 25(OH)D concentrations was not associated with markers of oxidative stress, endothelial function, inflammation, dyslipidaemia and bone health. This could suggest that low serum 25(OH)D concentrations is a consequence of kidney disease, but is not the cause for increased CVD markers (Theodoratou et al., 2014). Thus, low serum 25(OH)D concentrations is of limited importance for the development of CVD in calcium oxalate kidney stone patients

4.1 CONCLUSION

Taking the studies together, although there may be a possibility that vitamin D supplementation may improve cardiometabolic risk, as several observational studies have demonstrated that low vitamin D status is associated with cardiometabolic disease. However, there are limitations to applying observational data to clinical practice, as they are subject to confounding and do not draw causal inferences. Consistent with the finding of the present study, there is insufficient evidence from clinical trials recommending vitamin D supplementation for lowering CVD risk (Theodoratou et al., 2014). This is because high quality systematic reviews and meta-analyses of RCTs have not shown a consistent effect of vitamin D supplementation on cardiometabolic outcomes or that improving vitamin D status prevents non-musculoskeletal outcomes (Bolland, Grey, Gamble, & Reid, 2014; Theodoratou et al., 2014; Zuk et al., 2016). Thus, low vitamin D concentrations may be a marker of poor health rather than the cause of poor health (Theodoratou et al., 2014). It may be more beneficial to improve cardiovascular risk factors by consuming a healthy and balanced diet, maintaining a healthy body weight (BMI 20-25 kg/m²), avoiding smoking and a sedentary life style.

Future research particularly from RCTs are required to fill a gap for the RCT study by examining whether there is a dose-response relationship between vitamin D supplementation and cardiometabolic risk outcomes. Research using larger dose of vitamin D and a longer duration of supplementation may be required, as plasma 25(OH)D concentrations >75nmol/L are required for optimal extra-skeletal health (Bischoff-Ferrari, 2008). Further research is also required using overweight or obese males above 50 years and a serum/plasma 25(OH)D concentration below 25nmol/L. Research measuring VDR genotype polymorphisms may be significant in understanding the variations in individual response of the overweight and obese to oral vitamin D supplementation.

For the cross-sectional kidney stone study, possible future studies using a larger population with age-matched controls is required. Research investigating indices of atherosclerosis such as coronary artery calcium would likely provide distinct information regarding CVD risk in calcium oxalate kidney stone patients. Additionally, vitamin D supplementation in this population is required as this might

not only decrease serum PTH secretion (Lee et al., 2013), it would also improve VDR expression and activities that would in turn exert reno-protective effects by downregulating RAS activity. Vitamin D supplementation in this cohort would establish whether with optimal vitamin D status, CVD risk would be reduced. Finally, a study assessing circulating FGF-23 concentration to determine the extent of kidney dysfunction in calcium oxalate kidney stone patients' population is also required.

REFERENCES

- Aarts, E., van Groningen, L., Horst, R., Telting, D., van Sorge, A., Janssen, I., & de Boer, H. (2011). Vitamin D absorption: consequences of gastric bypass surgery. *Eur J Endocrinol*, 164(5), 827-832. doi:10.1530/eje-10-1126
- Abate, N., Chandalia, M., Cabo-Chan, A. V., Moe, O. W., & Sakhaee, K. (2004). The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. *Kidney Int*, 65(2), 386-392.
- Abbas, M. A. (2016). Physiological functions of Vitamin D in adipose tissue. *J Steroid Biochem Mol Biol*, 165(B), 369-381.
- Abdelbaqi-Salhab, M., Shalhub, S., & Morgan, M. B. (2003). A current review of the cutaneous manifestations of renal disease. *J Cutan Pathol*, 30(9), 527-538.
- Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., . . . Suda, T. (1981). Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. *Proc Natl Acad Sci U S A*, 78(8), 4990-4994.
- Adams, J. S., & Hewison, M. (2010). Update in vitamin D. *J Clin Endocrinol Metab*, 95(2), 471-478.
- Adorini, L., Penna, G., Giarratana, N., Roncari, A., Amuchastegui, S., Daniel, K. C., & Uskokovic, M. (2004). Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. *J Steroid Biochem Mol Biol.*, 89-90(1-5), 437-441. doi:10.1016/j.jsbmb.2004.03.013
- Afzal, S., Bojesen, S. E., & Nordestgaard, B. G. (2013). Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and metaanalysis. *Clin Chem*, 59(2), 381-391. doi:10.1373/clinchem.2012.193003
- Agbalalah, T., Hughes, S. F., Freeborn, E. J., & Mushtaq, S. Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: A systematic review. *J Steroid Biochem Mol Biol*. doi:<http://dx.doi.org/10.1016/j.jsbmb.2017.01.015>
- Aggarwal, K. P., Narula, S., Kakkar, M., & Tandon, C. (2013). Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. *Biomed Res Int*, 2013, 292953. doi:10.1155/2013/292953
- Ahmad, R., Al-Mass, A., Atizado, V., Al-Hubail, A., Al-Ghimlas, F., Al-Arouj, M., . . . Behbehani, K. (2012). Elevated expression of the toll like receptors 2 and 4

- in obese individuals: its significance for obesity-induced inflammation. *J Inflamm (Lond)*, 9(1), 48. doi:10.1186/1476-9255-9-48
- Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K. C., McCullough, M. L., Gallicchio, L., . . . Albanes, D. (2010). Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet*, 19(13), 2739-2745. doi:10.1093/hmg/ddq155
- Al-Daghri, N. M., Guerini, F. R., Al-Attas, O. S., Alokail, M. S., Alkharfy, K. M., Draz, H. M., . . . Mohammed, A. K. (2014). Vitamin D receptor gene polymorphisms are associated with obesity and inflammome activity. *PLoS One*, 9(7), e102141.
- Al-Dujaili, E. A., Munir, N., & Iniesta, R. R. (2016). Effect of vitamin D supplementation on cardiovascular disease risk factors and exercise performance in healthy participants: a randomized placebo-controlled preliminary study. *Ther Adv Endocrinol Metab*, 7(4), 153-165. doi:10.1177/2042018816653357
- Al Mheid, I., Patel, R., Murrow, J., Morris, A., Rahman, A., Fike, L., . . . Quyyumi, A. A. (2011). Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. *J Am Coll Cardiol*, 58(2), 186-192. doi:10.1016/j.jacc.2011.02.051
- Alberti, K. G. M. M., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diabetic medicine*, 23(5), 469-480.
- Alvarez, J. A., & Ashraf, A. (2009). Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol*, 2010.
- Amann, K. (2008). Media calcification and intima calcification are distinct entities in chronic kidney disease. *Clin J Am Soc Nephrol*, 3(6), 1599-1605. doi:10.2215/cjn.02120508
- Amling, M., Priemel, M., Holzmann, T., Chapin, K., Rueger, J. M., Baron, R., & Demay, M. B. (1999). Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology*, 140(11), 4982-4987. doi:10.1210/endo.140.11.7110

- Andersson, P., Rydberg, E., & Willenheimer, R. (2004). Primary hyperparathyroidism and heart disease--a review. *Eur Heart J*, 25(20), 1776-1787. doi:10.1016/j.ehj.2004.07.010
- Andress, D. L. (2005). Vitamin D treatment in chronic kidney disease. *Semin Dial*, 18(4), 315-321. doi:10.1111/j.1525-139X.2005.18408.x
- Andrukhova, O., Smorodchenko, A., Egerbacher, M., Streicher, C., Zeitz, U., Goetz, R., . . . Erben, R. G. (2014). FGF23 promotes renal calcium reabsorption through the TRPV5 channel. *EMBO J*, 33(3), 229-246. doi:10.1002/emboj.201284188
- Apukhovskaia, L. I., Khrestovaia, N. L., Antonenko, L. V., Omel'chenko, L. I., & Dotsenko, L. A. (1990). [Effect of varying vitamin D status in the body on intestinal absorption and metabolism of vitamin D in the rat liver]. *Ukr Biokhim Zh (1978)*, 62(6), 88-92.
- Argao, E. A., Heubi, J. E., Hollis, B. W., & Tsang, R. C. (1992). d-Alpha-tocopheryl polyethylene glycol-1000 succinate enhances the absorption of vitamin D in chronic cholestatic liver disease of infancy and childhood. *Pediatr Res*, 31(2), 146-150. doi:10.1203/00006450-199202000-00011
- Argraves, K. M., & Argraves, W. S. (2007). HDL serves as a S1P signaling platform mediating a multitude of cardiovascular effects. *J.lipid res.*, 48(11), 2325-2333.
- Armas, L. A., Hollis, B. W., & Heaney, R. P. (2004). Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab*, 89(11), 5387-5391. doi:10.1210/jc.2004-0360
- Armbricht, H. J., Zenser, T. V., & Davis, B. B. (1980). Effect of age on the conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 by kidney of rat. *J Clin Invest*, 66(5), 1118-1123. doi:10.1172/JCI109941
- Arnold, A. C., Okamoto, L. E., Gamboa, A., Shibao, C., Raj, S. R., Robertson, D., & Biaggioni, I. (2013). Angiotensin II, independent of plasma renin activity, contributes to the hypertension of autonomic failure. *Hypertension*, 61(3), 701-706.
- Aschner, P. (2010). Metabolic syndrome as a risk factor for diabetes. *Expert Rev Cardiovasc Ther*, 8(3), 407-412. doi:10.1586/erc.10.13
- Asemi, Z., Hashemi, T., Karamali, M., Samimi, M., & Esmailzadeh, A. (2013). Effects of vitamin D supplementation on glucose metabolism, lipid

- concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. *Am J Clin Nutr*, 98(6), 1425-1432. doi:10.3945/ajcn.113.072785
- Asemi, Z., Samimi, M., Tabassi, Z., Shakeri, H., & Esmailzadeh, A. (2013). Vitamin D supplementation affects serum high-sensitivity C-reactive protein, insulin resistance, and biomarkers of oxidative stress in pregnant women. *J Nutr*, 143(9), 1432-1438. doi:10.3945/jn.113.177550
- Ashraf, A. P., Alvarez, J. A., Dudenbostel, T., Calhoun, D., Griffin, R., Wang, X., . . . Gower, B. A. (2014). Associations between vascular health indices and serum total, free and bioavailable 25-hydroxyvitamin D in adolescents. *PLoS One*, 9(12), e114689.
- Ashraf, A. P., Alvarez, J. A., Gower, B. A., Saenz, K. H., & McCormick, K. L. (2011). Associations of serum 25-hydroxyvitamin D and components of the metabolic syndrome in obese adolescent females. *Obesity (Silver Spring)*, 19(11), 2214-2221. doi:10.1038/oby.2011.110
- Ashwell, M. (2011). Charts based on body mass index and waist-to-height ratio to assess the health risks of obesity: a review. *Open Obes J*, 3, 78-84.
- Badawi, A., Klip, A., Haddad, P., Cole, D. E., Bailo, B. G., El-Sohemy, A., & Karmali, M. (2010). Type 2 diabetes mellitus and inflammation: Prospects for biomarkers of risk and nutritional intervention. *Diabetes Metab Syndr Obes*, 3, 173-186.
- Bajaj, S., Singh, R. P., Dwivedi, N. C., Singh, K., Gupta, A., & Mathur, M. (2014). Vitamin D levels and microvascular complications in type 2 diabetes. *Indian J Endocrinol Metab*, 18(4), 537-541. doi:10.4103/2230-8210.137512
- Baker, R. G., Hayden, M. S., & Ghosh, S. (2011). NF-kappaB, inflammation, and metabolic disease. *Cell Metab*, 13(1), 11-22. doi:10.1016/j.cmet.2010.12.008
- Barter, P., Gotto, A. M., LaRosa, J. C., Maroni, J., Szarek, M., Grundy, S. M., . . . Treating to New Targets, I. (2007). HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med*, 357(13), 1301-1310. doi:10.1056/NEJMoa064278
- Barter, P. J., Nicholls, S., Rye, K.-A., Anantharamaiah, G., Navab, M., & Fogelman, A. M. (2004). Antiinflammatory properties of HDL. *Circ Res*, 95(8), 764-772.

- Bastard, J.-P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., . . . Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network*, 17(1), 4-12.
- Bates, B., Lennox, A., Prentice, A., Bates, C., Page, P., Nicholson, S., & Swan, G. (2014). National Diet and Nutrition Survey, results from years 1–4 of the rolling programme: London: Public Health England. www.gov.uk/government/uploads/system/uploads/attachment_data/file/310995/NDNS_Y1_to_4_UK_report.pdf.
- Baulmann, J., Schillings, U., Rickert, S., Uen, S., Düsing, R., Illyes, M., . . . Mengden, T. (2008). A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *Journal of hypertension*, 26(3), 523-528.
- Baulmann, J., Schillings, U., Rickert, S., Uen, S., Dusing, R., Illyes, M., . . . Mengden, T. (2008). A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *J Hypertens*, 26(3), 523-528. doi:10.1097/HJH.0b013e3282f314f7
- Bautista, D. S., Denstedt, J., Chambers, A. F., & Harris, J. F. (1996). Low-molecular-weight variants of osteopontin generated by serine proteinases in urine of patients with kidney stones. *J Cell Biochem*, 61(3), 402-409.
- Bei-Fan, Z. (2002). Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults: study on optimal cut-off points of body mass index and waist circumference in Chinese adults. *Asia Pac J Clin Nutr*, 11(s8), S685-S693.
- Bellia, A., Garcovich, C., D'Adamo, M., Lombardo, M., Tesauero, M., Donadel, G., . . . Lauro, R. (2013). Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. *Intern Emerg Med*, 8(1), 33-40.
- Bellosta, S., & Corsini, A. (2012). Statin drug interactions and related adverse reactions. *Expert opinion on drug safety*, 11(6), 933-946.
- Ben-Zvi, I., Aranow, C., Mackay, M., Stanevsky, A., Kamen, D. L., Marinescu, L. M., . . . Hardin, J. A. (2010). The impact of vitamin D on dendritic cell function in patients with systemic lupus erythematosus. *PLoS One*, 5(2), e9193.

- Benhusein, G. M., Mutch, E., Aburawi, S., & Williams, F. M. (2010). Genotoxic effect induced by hydrogen peroxide in human hepatoma cells using comet assay. *Libyan J Med*, 5. doi:10.3402/ljm.v5i0.4637
- Bentzon, J. F., Otsuka, F., Virmani, R., & Falk, E. (2014). Mechanisms of plaque formation and rupture. *Circ Res*, 114(12), 1852-1866.
- Berg, A. H., & Scherer, P. E. (2005). Adipose tissue, inflammation, and cardiovascular disease. *Circ Res*, 96(9), 939-949.
- Bergwitz, C., & Juppner, H. (2010). Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu Rev Med*, 61, 91-104. doi:10.1146/annurev.med.051308.111339
- Bhurosy, T., & Jeewon, R. (2013). Pitfalls of using body mass index (BMI) in assessment of obesity risk. *Curr. Res. Nutr. Food Sci.*, 1(1), 71-76.
- Bhutta, Z. A., Das, J. K., Rizvi, A., Gaffey, M. F., Walker, N., Horton, S., . . . Group, T. L. N. I. R. (2013). Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *The Lancet*, 382(9890), 452-477.
- Bian, K., Ishibashi, K., & Bukoski, R. D. (1996). 1,25(OH)₂D₃ modulates intracellular Ca²⁺ and force generation in resistance arteries. *Am J Physiol*, 270(1 Pt 2), H230-237.
- Biancuzzo, R. M., Young, A., Bibuld, D., Cai, M. H., Winter, M. R., Klein, E. K., . . . Holick, M. F. (2010). Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr*, 91(6), 1621-1626. doi:10.3945/ajcn.2009.27972
- Bikle, D. D. (2014). Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol*, 21(3), 319-329. doi:10.1016/j.chembiol.2013.12.016
- Bischoff-Ferrari, H. A. (2008). Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes *Sunlight, Vitamin D and Skin Cancer* (pp. 55-71): Springer.
- Bischoff-Ferrari, H. A., Shao, A., Dawson-Hughes, B., Hathcock, J., Giovannucci, E., & Willett, W. C. (2010). Benefit-risk assessment of vitamin D supplementation. *Osteoporos Int*, 21(7), 1121-1132. doi:10.1007/s00198-009-1119-3

- Black, L. J., Seamans, K. M., Cashman, K. D., & Kiely, M. (2012). An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *J Nutr*, 142(6), 1102-1108. doi:10.3945/jn.112.158014
- Blaha, M. J., Blumenthal, R. S., Brinton, E. A., Jacobson, T. A., & Cholesterol, N. L. A. T. o. N.-H. (2008). The importance of non-HDL cholesterol reporting in lipid management. *J. Clin. Lipidol.*, 2(4), 267-273.
- Blaine, J., Chonchol, M., & Levi, M. (2015). Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol*, 10(7), 1257-1272. doi:10.2215/cjn.09750913
- Bland, R., Markovic, D., Hills, C. E., Hughes, S. V., Chan, S. L., Squires, P. E., & Hewison, M. (2004). Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol*, 89-90(1-5), 121-125. doi:10.1016/j.jsbmb.2004.03.115
- Blum, M., Dolnikowski, G., Seyoum, E., Harris, S. S., Booth, S. L., Peterson, J., . . . Dawson-Hughes, B. (2008). Vitamin D(3) in fat tissue. *Endocrine*, 33(1), 90-94. doi:10.1007/s12020-008-9051-4
- Bolland, M. J., Grey, A., Gamble, G. D., & Reid, I. R. (2014). The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. *The lancet Diabetes & endocrinology*, 2(4), 307-320.
- Boonla, C., Hunapathed, C., Bovornpadungkitti, S., Poonpirome, K., Tungsanga, K., Sampatanukul, P., & Tosukhowong, P. (2008). Messenger RNA expression of monocyte chemoattractant protein-1 and interleukin-6 in stone-containing kidneys. *BJU Int*, 101(9), 1170-1177. doi:10.1111/j.1464-410X.2008.07461.x
- Boonla, C., Wunsuwan, R., Tungsanga, K., & Tosukhowong, P. (2007). Urinary 8-hydroxydeoxyguanosine is elevated in patients with nephrolithiasis. *Urol. Res.*, 35(4), 185-191.
- Borel, P. (2012). Genetic variations involved in interindividual variability in carotenoid status. *Mol Nutr Food Res*, 56(2), 228-240. doi:10.1002/mnfr.201100322
- Borel, P., Caillaud, D., & Cano, N. J. (2015). Vitamin D bioavailability: state of the art. *Crit Rev Food Sci Nutr*, 55(9), 1193-1205. doi:10.1080/10408398.2012.688897

- Borgi, L., McMullan, C., Wohlhueter, A., Curhan, G. C., Fisher, N. D., & Forman, J. P. (2016). Effect of Vitamin D on Endothelial Function: A Randomized, Double-Blind, Placebo-Controlled Trial. *Am J Hypertens*, hpw135.
- Bosworth, C., & de Boer, I. H. (2013). Impaired vitamin D metabolism in CKD. *Semin Nephrol*, 33(2), 158-168. doi:10.1016/j.semnephrol.2012.12.016
- Bosworth, C., Sachs, M. C., Duprez, D., Hoofnagle, A. N., Ix, J. H., Jacobs, D. R., Jr., . . . de Boer, I. H. (2013). Parathyroid hormone and arterial dysfunction in the multi-ethnic study of atherosclerosis. *Clin Endocrinol (Oxf)*, 79(3), 429-436. doi:10.1111/cen.12163
- Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A., Luderer, H. F., . . . Demay, M. (2008). Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*, 29(6), 726-776. doi:10.1210/er.2008-0004
- Bouillon, R., van Baelen, H., & de Moor, P. (1980). Comparative study of the affinity of the serum vitamin D-binding protein. *J Steroid Biochem*, 13(9), 1029-1034.
- Bouillon, R., Van Schoor, N. M., Gielen, E., Boonen, S., Mathieu, C., Vanderschueren, D., & Lips, P. (2013). Optimal vitamin D status: a critical analysis on the basis of evidence-based medicine. *J Clin Endocrinol Metab*, 98(8), E1283-1304. doi:10.1210/jc.2013-1195
- Bray, G. A., & Bellanger, T. (2006). Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. *Endocrine*, 29(1), 109-117. doi:10.1385/ENDO:29:1:109
- Bray, G. A., Clearfield, M. B., Fintel, D. J., & Nelinson, D. S. (2009). Overweight and obesity: the pathogenesis of cardiometabolic risk. *Clin Cornerstone*, 9(4), 30-40; discussion 41-32.
- Bressendorff, I., Brandi, L., Schou, M., Nygaard, B., Frandsen, N. E., Rasmussen, K., . . . Hansen, D. (2016). The Effect of High Dose Cholecalciferol on Arterial Stiffness and Peripheral and Central Blood Pressure in Healthy Humans: A Randomized Controlled Trial. *PLoS One*, 11(8), e0160905. doi:10.1371/journal.pone.0160905
- Brewer, L. C., Michos, E. D., & Reis, J. P. (2011). Vitamin D in atherosclerosis, vascular disease, and endothelial function. *Curr Drug Targets*, 12(1), 54-60.

- Brito Galvao, J. F., Nagode, L. A., Schenck, P. A., & Chew, D. J. (2013). Calcitriol, calcidiol, parathyroid hormone, and fibroblast growth factor-23 interactions in chronic kidney disease. *J. Vet. Emerg. and Crit. Care.*, 23(2), 134-162.
- Brunet, P., Gondouin, B., Duval-Sabatier, A., Dou, L., Cerini, C., Dignat-George, F., . . . Burtey, S. (2011). Does uremia cause vascular dysfunction? *Kidney Blood Press Res*, 34(4), 284-290. doi:10.1159/000327131
- Buckley, D. I., Fu, R., Freeman, M., Rogers, K., & Helfand, M. (2009). C-reactive protein as a risk factor for coronary heart disease: a systematic review and meta-analyses for the U.S. Preventive Services Task Force. *Ann Intern Med*, 151(7), 483-495.
- Buizert, P. J., van Schoor, N. M., Simsek, S., Lips, P., Heijboer, A. C., den Heijer, M., . . . Eekhoff, E. M. (2013). PTH: a new target in arteriosclerosis? *J Clin Endocrinol Metab*, 98(10), E1583-1590. doi:10.1210/jc.2013-1621
- Burgaz, A., Orsini, N., Larsson, S. C., & Wolk, A. (2011). Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J Hypertens*, 29(4), 636-645. doi:10.1097/HJH.0b013e32834320f9
- Byon, C. H., Javed, A., Dai, Q., Kappes, J. C., Clemens, T. L., Darley-Usmar, V. M., . . . Chen, Y. (2008). Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J Biol Chem*, 283(22), 15319-15327.
- Byrdwell, W. C., Devries, J., Exler, J., Harnly, J. M., Holden, J. M., Holick, M. F., . . . Wolf, W. R. (2008). Analyzing vitamin D in foods and supplements: methodologic challenges. *Am J Clin Nutr*, 88(2), 554S-557S.
- Caballero, B. (2007). The global epidemic of obesity: an overview. *Epidemiol Rev*, 29, 1-5. doi:10.1093/epirev/mxm012
- Calabresi, L., Gomasaschi, M., Villa, B., Omoboni, L., Dmitrieff, C., & Franceschini, G. (2002). Elevated soluble cellular adhesion molecules in subjects with low HDL-cholesterol. *Arterioscler Thromb Vasc Biol*, 22(4), 656-661.
- Caldwell, M. M., & Flint, S. D. (1994). Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Clim change*, 28(4), 375-394.
- Calton, E. K., Keane, K. N., Newsholme, P., & Soares, M. J. (2015). The Impact of Vitamin D Levels on Inflammatory Status: A Systematic Review of Immune Cell Studies. *PLoS One*, 10(11), e0141770. doi:10.1371/journal.pone.0141770

- Canaff, L., & Hendy, G. N. (2002). Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biol Chem*, 277(33), 30337-30350. doi:10.1074/jbc.M201804200
- Cantorna, M. T., Zhu, Y., Froicu, M., & Wittke, A. (2004). Vitamin D status, 1,25-dihydroxyvitamin D₃, and the immune system. *Am J Clin Nutr*, 80(6 Suppl), 1717S-1720S.
- Carlberg, C., & Molnar, F. (2012). Current status of vitamin D signaling and its therapeutic applications. *Curr Top Med Chem*, 12(6), 528-547.
- Carrara, D., Bernini, M., Bacca, A., Rugani, I., Duranti, E., Virdis, A., . . . Bernini, G. (2014). Cholecalciferol administration blunts the systemic renin-angiotensin system in essential hypertensives with hypovitaminosis D. *J Renin Angiotensin Aldosterone Syst*, 15(1), 82-87. doi:10.1177/1470320312471149
- Carter, G. D. (2009). 25-Hydroxyvitamin D assays: the quest for accuracy. *Clin Chem*, 55(7), 1300-1302. doi:10.1373/clinchem.2009.125906
- Carter, G. D., Berry, J. L., Gunter, E., Jones, G., Jones, J. C., Makin, H. L., . . . Wheeler, M. J. (2010). Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays. *J Steroid Biochem Mol Biol*, 121(1-2), 176-179. doi:10.1016/j.jsbmb.2010.03.033
- Castrillo, A., Joseph, S. B., Vaidya, S. A., Haberland, M., Fogelman, A. M., Cheng, G., & Tontonoz, P. (2003). Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Molecular cell*, 12(4), 805-816.
- Cecelja, M., & Chowienzyk, P. (2012). Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis*, 1(4). doi:10.1258/cvd.2012.012016
- Cecelja, M., Jiang, B., Spector, T. D., & Chowienzyk, P. (2012). Progression of central pulse pressure over 1 decade of aging and its reversal by nitroglycerin: a twin study. *J Am Coll Cardiol*, 59(5), 475-483.
- Chagas, C. E., Borges, M. C., Martini, L. A., & Rogero, M. M. (2012). Focus on vitamin D, inflammation and type 2 diabetes. *Nutrients*, 4(1), 52-67. doi:10.3390/nu4010052
- Chait, A., & Bornfeldt, K. E. (2009). Diabetes and atherosclerosis: is there a role for hyperglycemia? *J lipid Res*, 50(Supplement), S335-S339.

- Chandler, P., Scott, J., Drake, B., Ng, K., Chan, A., Hollis, B., . . . Bennett, G. (2015). Impact of vitamin D supplementation on adiposity in African-Americans. *Nutr Diabetes*, 5(1), e147.
- Chapuy, M. C., Preziosi, P., Maamer, M., Arnaud, S., Galan, P., Hercberg, S., & Meunier, P. J. (1997). Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int*, 7(5), 439-443.
- Chaudhuri, J. R., Mridula, K. R., Anamika, A., Boddu, D. B., Misra, P. K., Lingaiah, A., . . . Bandaru, V. S. (2013). Deficiency of 25-Hydroxyvitamin D and Dyslipidemia in Indian Subjects. *J Lipids*, 2013, 623420. doi:10.1155/2013/623420
- Chel, V., Wijnhoven, H. A., Smit, J. H., Ooms, M., & Lips, P. (2008). Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int*, 19(5), 663-671. doi:10.1007/s00198-007-0465-2
- Chen, P., Hu, P., Xie, D., Qin, Y., Wang, F., & Wang, H. (2010). Meta-analysis of vitamin D, calcium and the prevention of breast cancer. *Breast Cancer Res Treat*, 121(2), 469-477. doi:10.1007/s10549-009-0593-9
- Chen, T. C., Chimeh, F., Lu, Z., Mathieu, J., Person, K. S., Zhang, A., . . . Holick, M. F. (2007). Factors that influence the cutaneous synthesis and dietary sources of vitamin D. *Arch Biochem Biophys*, 460(2), 213-217. doi:10.1016/j.abb.2006.12.017
- Cheng, S., Massaro, J. M., Fox, C. S., Larson, M. G., Keyes, M. J., McCabe, E. L., . . . Wang, T. J. (2010). Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes*, 59(1), 242-248. doi:10.2337/db09-1011
- Chesney, M. A., Ickovics, J., Chambers, D., Gifford, A., Neidig, J., Zwickl, B., . . . GROUP, A. W. G. O. T. O. C. O. T. A. A. C. T. (2000). Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG adherence instruments. *AIDS care*, 12(3), 255-266.
- Chillaron, J., Font-Llitjos, M., Fort, J., Zorzano, A., Goldfarb, D. S., Nunes, V., & Palacin, M. (2010). Pathophysiology and treatment of cystinuria. *Nat Rev Nephrol*, 6(7), 424-434. doi:10.1038/nrneph.2010.69
- Chitalia, N., Recio-Mayoral, A., Kaski, J. C., & Banerjee, D. (2012). Vitamin D deficiency and endothelial dysfunction in non-dialysis chronic kidney disease

- patients. *Atherosclerosis*, 220(1), 265-268.
doi:10.1016/j.atherosclerosis.2011.10.023
- Chiu, K. C., Chu, A., Go, V. L., & Saad, M. F. (2004). Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr*, 79(5), 820-825.
- Chlebowski, R. T. (2011). Vitamin D and breast cancer: interpreting current evidence. *Breast Cancer Res*, 13(4), 217. doi:10.1186/bcr2846
- Chonchol, M., & Scragg, R. (2007). 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. *Kidney Int*, 71(2), 134-139. doi:10.1038/sj.ki.5002002
- Christakos, S., Dhawan, P., Liu, Y., Peng, X., & Porta, A. (2003). New insights into the mechanisms of vitamin D action. *J Cell Biochem*, 88(4), 695-705. doi:10.1002/jcb.10423
- Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L., & Carmeliet, G. (2016). Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev*, 96(1), 365-408. doi:10.1152/physrev.00014.2015
- Christakos, S., Lieben, L., Masuyama, R., & Carmeliet, G. (2014). Vitamin D endocrine system and the intestine. *Bonekey Rep*, 3, 496. doi:10.1038/bonekey.2013.230
- Christen, T., Trompet, S., Gast, K., Rensen, P., van Dijk, J. W., Lamb, H., . . . Le Cessie, S. (2016). The role of inflammation in the association between adiposity and subclinical atherosclerosis. *Atherosclerosis*, 252, e261.
- Chun, R. F. (2012). New perspectives on the vitamin D binding protein. *Cell Biochem Funct*, 30(6), 445-456. doi:10.1002/cbf.2835
- Chun, R. F., Peercy, B. E., Orwoll, E. S., Nielson, C. M., Adams, J. S., & Hewison, M. (2014). Vitamin D and DBP: the free hormone hypothesis revisited. *J Steroid Biochem Mol Biol*, 144 Pt A, 132-137. doi:10.1016/j.jsbmb.2013.09.012
- Clore, J. N., Post, E. P., Bailey, D. J., Nestler, J. E., & Blackard, W. G. (1992). Evidence for increased liver glycogen in patients with noninsulin-dependent diabetes mellitus after a 3-day fast. *J Clin Endocrinol Metab*, 74(3), 660-666.
- Cockerill, G. W., Huehns, T. Y., Weerasinghe, A., Stocker, C., Lerch, P. G., Miller, N. E., & Haskard, D. O. (2001). Elevation of plasma high-density lipoprotein

- concentration reduces interleukin-1–induced expression of E-selectin in an in vivo model of acute inflammation. *Circulation*, 103(1), 108-112.
- Codoner-Franch, P., Tavaréz-Alonso, S., Simo-Jorda, R., Laporta-Martin, P., Carratala-Calvo, A., & Alonso-Iglesias, E. (2012). Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. *J Pediatr*, 161(5), 848-854. doi:10.1016/j.jpeds.2012.04.046
- Coelho, I. M., Andrade, L. D., Saldanha, L., Diniz, E. T., Griz, L., & Bandeira, F. (2010). Bioavailability of vitamin D3 in non-oily capsules: the role of formulated compounds and implications for intermittent replacement. *Arg Bras Endocrinol Metabol*, 54(2), 239-243.
- Cohen-Lahav, M., Douvdevani, A., Chaimovitz, C., & Shany, S. (2007). The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. *J Steroid Biochem Mol Biol*, 103(3-5), 558-562. doi:10.1016/j.jsbmb.2006.12.093
- Cohen-Lahav, M., Shany, S., Tobvin, D., Chaimovitz, C., & Douvdevani, A. (2006). Vitamin D decreases NFkappaB activity by increasing IkappaBalpha levels. *Nephrol Dial Transplant*, 21(4), 889-897. doi:10.1093/ndt/gfi254
- Colaci, M., Giuggioli, D., Manfredi, A., Sebastiani, M., Coppi, F., Rossi, R., & Ferri, C. (2012). Aortic pulse wave velocity measurement in systemic sclerosis patients. *Reumatismo*, 64(6), 360-367.
- Compston, J. E., Merrett, A. L., Hammett, F. G., & Magill, P. (1981). Comparison of the appearance of radiolabelled vitamin D3 and 25-hydroxy-vitamin D3 in the chylomicron fraction of plasma after oral administration in man. *Clin Sci (Lond)*, 60(2), 241-243.
- Cooper, J. D., Smyth, D. J., Walker, N. M., Stevens, H., Burren, O. S., Wallace, C., . . . Todd, J. A. (2011). Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes*, 60(5), 1624-1631. doi:10.2337/db10-1656
- Cordero, J. B., Cozzolino, M., Lu, Y., Vidal, M., Slatopolsky, E., Stahl, P. D., . . . Dusso, A. (2002). 1,25-Dihydroxyvitamin D down-regulates cell membrane growth- and nuclear growth-promoting signals by the epidermal growth factor receptor. *J Biol Chem*, 277(41), 38965-38971. doi:10.1074/jbc.M203736200

- Covic, A., Voroneanu, L., & Goldsmith, D. (2010). The effects of vitamin D therapy on left ventricular structure and function - are these the underlying explanations for improved CKD patient survival? *Nephron Clin Pract*, 116(3), c187-195. doi:10.1159/000317198
- Cozzolino, M., Brancaccio, D., Gallieni, M., & Slatopolsky, E. (2005). Pathogenesis of vascular calcification in chronic kidney disease. *Kidney Int*, 68(2), 429-436. doi:10.1111/j.1523-1755.2005.00421.x
- Crowe, F. L., Steur, M., Allen, N. E., Appleby, P. N., Travis, R. C., & Key, T. J. (2011). Plasma concentrations of 25-hydroxyvitamin D in meat eaters, fish eaters, vegetarians and vegans: results from the EPIC-Oxford study. *Public Health Nutr*, 14(2), 340-346. doi:10.1017/s1368980010002454
- Cuppari, L., Carvalho, A. B., & Draibe, S. A. (2008). Vitamin D status of chronic kidney disease patients living in a sunny country. *J Ren Nutr*, 18(5), 408-414. doi:10.1053/j.jrn.2008.05.004
- Cuppari, L., & Garcia-Lopes, M. G. (2009). Hypovitaminosis D in chronic kidney disease patients: prevalence and treatment. *J Ren Nutr*, 19(1), 38-43. doi:10.1053/j.jrn.2008.10.005
- Da Costa, L. A., Arora, P., Garcia-Bailo, B., Karmali, M., El-Sohemy, A., & Badawi, A. (2012). The association between obesity, cardiometabolic disease biomarkers, and innate immunity-related inflammation in Canadian adults. *Diabetes Metab Syndr Obes*, 5, 347-355. doi:10.2147/DMSO.S35115
- Dalan, R., Liew, H., Assam, P. N., Chan, E. S., Siddiqui, F. J., Tan, A. W., . . . Leow, M. K. (2016). A randomised controlled trial evaluating the impact of targeted vitamin D supplementation on endothelial function in type 2 diabetes mellitus: The DIMENSION trial. *Diab Vasc Dis Res*, 13(3), 192-200. doi:10.1177/1479164115621667
- Dalan, R., Liew, H., Tan, W. K. A., Chew, D. E., & Leow, M. K.-S. (2014). Vitamin D and the endothelium: basic, translational and clinical research updates. *IJC Metabolic & Endocrine*, 4, 4-17.
- Dale, O., & Salo, M. (1996). The Helsinki Declaration, research guidelines and regulations: present and future editorial aspects. *Acta Anaesthesiol Scand*, 40(7), 771-772.

- Dalgard, C., Petersen, M. S., Weihe, P., & Grandjean, P. (2011). Vitamin D status in relation to glucose metabolism and type 2 diabetes in septuagenarians. *Diabetes Care*, 34(6), 1284-1288. doi:10.2337/dc10-2084
- Dalgård, C., Petersen, M. S., Weihe, P., & Grandjean, P. (2011). Vitamin D status in relation to glucose metabolism and type 2 diabetes in septuagenarians. *Diabetes Care*, 34(6), 1284-1288.
- Danik, J. S., & Manson, J. E. (2012). Vitamin D and cardiovascular disease. *Current treatment options in cardiovascular medicine*, 14(4), 414-424.
- Dardamanis, M. (2013). Pathomechanisms of nephrolithiasis. *Hippokratia*, 17(2), 100-107.
- Darling, A. L., Hart, K. H., Macdonald, H. M., Horton, K., Kang'ombe, A. R., Berry, J. L., & Lanham-New, S. A. (2013). Vitamin D deficiency in UK South Asian Women of childbearing age: a comparative longitudinal investigation with UK Caucasian women. *Osteoporos Int*, 24(2), 477-488. doi:10.1007/s00198-012-1973-2
- Datta, S., Alfaham, M., Davies, D. P., Dunstan, F., Woodhead, S., Evans, J., & Richards, B. (2002). Vitamin D deficiency in pregnant women from a non-European ethnic minority population--an interventional study. *BJOG*, 109(8), 905-908.
- Davidson, M. B., Duran, P., Lee, M. L., & Friedman, T. C. (2013). High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. *Diabetes Care*, 36(2), 260-266.
- Davies, J. M., Bailey, M. A., Griffin, K. J., & Scott, D. J. A. (2012). Pulse wave velocity and the non-invasive methods used to assess it: Complior, SphygmoCor, Arteriograph and Vicorder. *Vascular*, 20(6), 342-349.
- Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation*, 109(23 Suppl 1), Ii27-32. doi:10.1161/01.CIR.0000131515.03336.f8
- Dawodu, A., & Akinbi, H. (2013). Vitamin D nutrition in pregnancy: current opinion. *Int J Womens Health*, 5, 333-343. doi:10.2147/ijwh.s34032
- Dawson, C. H., & Tomson, C. R. (2012). Kidney stone disease: pathophysiology, investigation and medical treatment. *Clin Med (Lond)*, 12(5), 467-471.
- de Almeida-Pititto, B., Ribeiro-Filho, F. F., Bittencourt, M. S., Lotufo, P. A., Bensenor, I., & Ferreira, S. R. (2016). Usefulness of circulating E-selectin to

- early detection of the atherosclerotic process in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). *Diabetol Metab Syndr*, 8, 19. doi:10.1186/s13098-016-0133-9
- de Almeida, J. P., Liberatti, L. S., Barros, F. E., Kallaur, A. P., Lozovoy, M. A., Scavuzzi, B. M., . . . Simao, A. N. (2016). Profile of oxidative stress markers is dependent on vitamin D levels in patients with chronic hepatitis C. *Nutrition*, 32(3), 362-367. doi:10.1016/j.nut.2015.09.016
- Deb, D. K., Chen, Y., Zhang, Z., Zhang, Y., Szeto, F. L., Wong, K. E., . . . Li, Y. C. (2009). 1, 25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF- κ B pathway. *American Journal of Physiology-Renal Physiology*, 296(5), F1212-F1218.
- Deeb, K. K., Trump, D. L., & Johnson, C. S. (2007). Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer*, 7(9), 684-700. doi:10.1038/nrc2196
- Deleskog, A., Hilding, A., Brismar, K., Hamsten, A., Efendic, S., & Ostenson, C. G. (2012). Low serum 25-hydroxyvitamin D level predicts progression to type 2 diabetes in individuals with prediabetes but not with normal glucose tolerance. *Diabetologia*, 55(6), 1668-1678. doi:10.1007/s00125-012-2529-x
- DeLuca, H. F. (2008). Evolution of our understanding of vitamin D. *Nutr Rev*, 66(10 Suppl 2), S73-87. doi:10.1111/j.1753-4887.2008.00105.x
- Deng, X., Cheng, J., & Shen, M. (2016). Vitamin D improves diabetic nephropathy in rats by inhibiting renin and relieving oxidative stress. *J Endocrinol Invest*, 39(6), 657-666. doi:10.1007/s40618-015-0414-4
- Deng, X. R., Zhang, Y. F., Wang, T. G., Xu, B. H., Sun, J. C., Zhao, L. B., . . . Lu, J. L. (2014). Serum calcium level is associated with brachial-ankle pulse wave velocity in middle-aged and elderly Chinese. *Biomed Environ Sci*, 27(8), 594-600. doi:10.3967/bes2014.091
- Department of Health. (2013). Nutrient analysis of fish and fish products and nutrient analysis of eggs summary report. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/167921/Nutrient_analysis_of_fish_and_fish_products_-_Summary_Report.pdf

- Diamond, T., Wong, Y., & Golombick, T. (2013). Effect of oral cholecalciferol 2,000 versus 5,000 IU on serum vitamin D, PTH, bone and muscle strength in patients with vitamin D deficiency. *Osteoporis Int*, 24(3), 1101-1105.
- Dickie, L. J., Church, L. D., Coulthard, L. R., Mathews, R. J., Emery, P., & McDermott, M. F. (2010). Vitamin D3 down-regulates intracellular Toll-like receptor 9 expression and Toll-like receptor 9-induced IL-6 production in human monocytes. *Rheumatology (Oxford)*, 49(8), 1466-1471. doi:10.1093/rheumatology/keq124
- Ding, C., Gao, D., Wilding, J., Trayhurn, P., & Bing, C. (2012). Vitamin D signalling in adipose tissue. *Br J Nutr*, 108(11), 1915-1923. doi:10.1017/s0007114512003285
- Ding, C., Parameswaran, V., Blizzard, L., Burgess, J., & Jones, G. (2010). Not a simple fat-soluble vitamin: Changes in serum 25-(OH)D levels are predicted by adiposity and adipocytokines in older adults. *J Intern Med*, 268(5), 501-510. doi:10.1111/j.1365-2796.2010.02267.x
- Ding, C., Wilding, J. P., & Bing, C. (2013). 1, 25-dihydroxyvitamin D 3 Protects against Macrophage-Induced Activation of NFκB and MAPK Signalling and Chemokine Release in Human Adipocytes. *PLoS One*, 8(4), e61707.
- Dinour, D., Beckerman, P., Ganon, L., Tordjman, K., Eisenstein, Z., & Holtzman, E. J. (2013). Loss-of-function mutations of CYP24A1, the vitamin D 24-hydroxylase gene, cause long-standing hypercalciuric nephrolithiasis and nephrocalcinosis. *J Urol*, 190(2), 552-557. doi:10.1016/j.juro.2013.02.3188
- Djordjevic, V. B., Zvezdanovic, L., & Cosic, V. (2008). [Oxidative stress in human diseases]. *Srp Arh Celok Lek*, 136 Suppl 2, 158-165.
- Dorian, H. H., Rez, P., & Drach, G. W. (1996). Evidence for aggregation in oxalate stone formation: atomic force and low voltage scanning electron microscopy. *J Urol*, 156(5), 1833-1837.
- Drincic, A. T., Armas, L. A., Van Diest, E. E., & Heaney, R. P. (2012). Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring)*, 20(7), 1444-1448. doi:10.1038/oby.2011.404
- Dunlop, T. W., Vaisanen, S., Frank, C., Molnar, F., Sinkkonen, L., & Carlberg, C. (2005). The human peroxisome proliferator-activated receptor delta gene is a primary target of 1alpha,25-dihydroxyvitamin D3 and its nuclear receptor. *J Mol Biol*, 349(2), 248-260. doi:10.1016/j.jmb.2005.03.060

- Dusso, A. S., Brown, A. J., & Slatopolsky, E. (2005). Vitamin D. *Am J Physiol Renal Physiol*, 289(1), F8-28. doi:10.1152/ajprenal.00336.2004
- Dzau, V. J. (2001). Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. *Hypertension*, 37(4), 1047-1052.
- Earthman, C. P., Beckman, L. M., Masodkar, K., & Sibley, S. D. (2012). The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int J Obes (Lond)*, 36(3), 387-396. doi:10.1038/ijo.2011.119
- Edson, K. Z., Prasad, B., Unadkat, J. D., Suhara, Y., Okano, T., Guengerich, F. P., & Rettie, A. E. (2013). Cytochrome P450-dependent catabolism of vitamin K: ω -hydroxylation catalyzed by human CYP4F2 and CYP4F11. *Biochemistry*, 52(46), 8276-8285.
- Eisner, B. H., Thavaseelan, S., Sheth, S., Haleblan, G., & Pareek, G. (2012). Relationship between serum vitamin D and 24-hour urine calcium in patients with nephrolithiasis. *Urology*, 80(5), 1007-1010. doi:10.1016/j.urology.2012.04.041
- Elamin, M. B., Abu Elnour, N. O., Elamin, K. B., Fatourehchi, M. M., Alkatib, A. A., Almandoz, J. P., . . . Montori, V. M. (2011). Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab*, 96(7), 1931-1942. doi:10.1210/jc.2011-0398
- Elkoushy, M. A., Sabbagh, R., Unikowsky, B., & Andonian, S. (2012). Prevalence and metabolic abnormalities of vitamin D-inadequate patients presenting with urolithiasis to a tertiary stone clinic. *Urology*, 79(4), 781-785. doi:10.1016/j.urology.2011.09.004
- Ellero, S., Chakhtoura, G., Barreau, C., Langouët, S., Benelli, C., Penicaud, L., . . . De Waziers, I. (2010). Xenobiotic-metabolizing cytochromes p450 in human white adipose tissue: expression and induction. *Drug Metab Dispos*, 38(4), 679-686.
- Endemann, D. H., & Schiffrin, E. L. (2004). Endothelial dysfunction. *J Am Soc Nephrol*, 15(8), 1983-1992. doi:10.1097/01.ASN.0000132474.50966.DA
- Engelman, C. D., Fingerlin, T. E., Langefeld, C. D., Hicks, P. J., Rich, S. S., Wagenknecht, L. E., . . . Norris, J. M. (2008). Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in

- Hispanic and African Americans. *J Clin Endocrinol Metab*, 93(9), 3381-3388. doi:10.1210/jc.2007-2702
- Ennis, J. L., Worcester, E. M., Coe, F. L., & Sprague, S. M. (2016). Current recommended 25-hydroxyvitamin D targets for chronic kidney disease management may be too low. *J Nephrol*, 29(1), 63-70. doi:10.1007/s40620-015-0186-0
- Esteve, E., Ricart, W., & Fernandez-Real, J. M. (2005). Dyslipidemia and inflammation: an evolutionary conserved mechanism. *Clin Nutr*, 24(1), 16-31.
- European Food Safety Authority. (2012). Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. *EFSA*, 10(7), 2813.
- Evan, A. P., Lingeman, J. E., Worcester, E. M., Bledsoe, S. B., Sommer, A. J., Williams, J. C., Jr., . . . Coe, F. L. (2010). Renal histopathology and crystal deposits in patients with small bowel resection and calcium oxalate stone disease. *Kidney Int*, 78(3), 310-317. doi:10.1038/ki.2010.131
- Fadini, G. P., Miorin, M., Facco, M., Bonamico, S., Baesso, I., Grego, F., . . . Agostini, C. (2005). Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol*, 45(9), 1449-1457.
- Fahal, I. H. (2014). Uraemic sarcopenia: aetiology and implications. *Nephrol Dial Transplant*, 29(9), 1655-1665. doi:10.1093/ndt/gft070
- Fakheri, R. J., & Goldfarb, D. S. (2011). Ambient temperature as a contributor to kidney stone formation: implications of global warming. *Kidney Int*, 79(11), 1178-1185. doi:10.1038/ki.2011.76
- Farell, G., Huang, E., Kim, S. Y., Horstkorte, R., & Lieske, J. C. (2004). Modulation of proliferating renal epithelial cell affinity for calcium oxalate monohydrate crystals. *J Am Soc Nephrol*, 15(12), 3052-3062. doi:10.1097/01.ASN.0000144205.49134.64
- Farraye, F. A., Nimitphong, H., Stucchi, A., Dendrinos, K., Boulanger, A. B., Vijjeswarapu, A., . . . Holick, M. F. (2011). Use of a novel vitamin D bioavailability test demonstrates that vitamin D absorption is decreased in patients with quiescent Crohn's disease. *Inflamm Bowel Dis*, 17(10), 2116-2121. doi:10.1002/ibd.21595

- Farro, I., Bia, D., Zócalo, Y., Torrado, J., Farro, F., Florio, L., . . . Armentano, R. L. (2012). Pulse wave velocity as marker of preclinical arterial disease: reference levels in a uruguayan population considering wave detection algorithms, path lengths, aging, and blood pressure. *International journal of hypertension*, 2012.
- Fasano, J. M., & Khan, S. R. (2001). Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. *Kidney Int*, 59(1), 169-178. doi:10.1046/j.1523-1755.2001.00477.x
- Fattah, H., Hambaroush, Y., & Goldfarb, D. S. (2014). Cystine nephrolithiasis. *Transl Androl Urol*, 3(3), 228-233. doi:10.3978/j.issn.2223-4683.2014.07.04
- Ferder, M., Insera, F., Manucha, W., & Ferder, L. (2013). The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. *Am J Physiol Cell Physiol*, 304(11), C1027-1039. doi:10.1152/ajpcell.00403.2011
- Fernandez-Alonso, A. M., Dionis-Sanchez, E. C., Chedraui, P., Gonzalez-Salmeron, M. D., Perez-Lopez, F. R., Spanish Vitamin, D., & Women's Health Research, G. (2012). First-trimester maternal serum 25-hydroxyvitamin D(3) status and pregnancy outcome. *Int J Gynaecol Obstet*, 116(1), 6-9. doi:10.1016/j.ijgo.2011.07.029
- Flannigan, R., Choy, W. H., Chew, B., & Lange, D. (2014). Renal struvite stones--pathogenesis, microbiology, and management strategies. *Nat Rev Urol*, 11(6), 333-341. doi:10.1038/nrurol.2014.99
- Flores, M. (2005). A role of vitamin D in low-intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus? *Nutr Res Rev*, 18(2), 175-182. doi:10.1079/nrr2005104
- Folsom, A. R., Alonso, A., Misialek, J. R., Michos, E. D., Selvin, E., Eckfeldt, J. H., . . . Lutsey, P. L. (2014). Parathyroid hormone concentration and risk of cardiovascular diseases: the Atherosclerosis Risk in Communities (ARIC) study. *Am Heart J*, 168(3), 296-302. doi:10.1016/j.ahj.2014.04.017
- Foltyn, V. N., Bendikov, I., De Miranda, J., Panizzutti, R., Dumin, E., Shleper, M., . . . Wolosker, H. (2005). Serine racemase modulates intracellular D-serine levels through an alpha,beta-elimination activity. *J Biol Chem*, 280(3), 1754-1763. doi:10.1074/jbc.M405726200

- Forman, J. P., Scott, J. B., Ng, K., Drake, B. F., Suarez, E. G., Hayden, D. L., . . . Chan, A. T. (2013). Effect of vitamin D supplementation on blood pressure in blacks. *Hypertension*, *61*(4), 779-785. doi:10.1161/hypertensionaha.111.00659
- Forouhi, N. G., Luan, J. a., Cooper, A., Boucher, B. J., & Wareham, N. J. (2008). Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance the medical research council ely prospective study 1990–2000. *Diabetes*, *57*(10), 2619-2625.
- Forouhi, N. G., Ye, Z., Rickard, A. P., Khaw, K. T., Luben, R., Langenberg, C., & Wareham, N. J. (2012). Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia*, *55*(8), 2173-2182. doi:10.1007/s00125-012-2544-y
- Forrest, K. Y., & Stuhldreher, W. L. (2011). Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res*, *31*(1), 48-54. doi:10.1016/j.nutres.2010.12.001
- Förstermann, U. (2010). Nitric oxide and oxidative stress in vascular disease. *Pflügers Archiv-Eur J Physiol*, *459*(6), 923-939.
- Forsythe, L. K., Livingstone, M. B., Barnes, M. S., Horigan, G., McSorley, E. M., Bonham, M. P., . . . Wallace, J. M. (2012). Effect of adiposity on vitamin D status and the 25-hydroxycholecalciferol response to supplementation in healthy young and older Irish adults. *Br J Nutr*, *107*(1), 126-134. doi:10.1017/S0007114511002662
- Franklin, S. S., Larson, M. G., Khan, S. A., Wong, N. D., Leip, E. P., Kannel, W. B., & Levy, D. (2001). Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation*, *103*(9), 1245-1249.
- Frederich, R., Alexander, J. H., Fiedorek, F. T., Donovan, M., Berglind, N., Harris, S., . . . Mahaffey, K. W. (2015). A systematic assessment of cardiovascular outcomes in the saxagliptin drug development program for type 2 diabetes. *Postgraduate medicine*.

- Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, 18(6), 499-502.
- Fuentes Q, E., Fuentes Q, F., Andrés, V., Pello, O. M., de Mora, J. F., & Palomo G, I. (2013). Role of platelets as mediators that link inflammation and thrombosis in atherosclerosis. *Platelets*, 24(4), 255-262.
- Fukuda, N., Tanaka, H., Tominaga, Y., Fukagawa, M., Kurokawa, K., & Seino, Y. (1993). Decreased 1,25-dihydroxyvitamin D3 receptor density is associated with a more severe form of parathyroid hyperplasia in chronic uremic patients. *J Clin Invest*, 92(3), 1436-1443. doi:10.1172/jci116720
- Gal-Moscovici, A., & Sprague, S. M. (2007). Role of vitamin D deficiency in chronic kidney disease. *J Bone Miner Res*, 22(S2), V91-V94.
- Garland, C. F., French, C. B., Baggerly, L. L., & Heaney, R. P. (2011). Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. *Anticancer Res*, 31(2), 607-611.
- Gayoso-Diz, P., Otero-González, A., Rodriguez-Alvarez, M. X., Gude, F., García, F., De Francisco, A., & Quintela, A. G. (2013). Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC endocrine disorders*, 13(1), 1.
- Gersch, C., Palii, S. P., Kim, K. M., Angerhofer, A., Johnson, R. J., & Henderson, G. N. (2008). Inactivation of nitric oxide by uric acid. *Nucleosides Nucleotides Nucleic Acids*, 27(8), 967-978. doi:10.1080/15257770802257952
- Ginde, A. A., Mansbach, J. M., & Camargo, C. A., Jr. (2009). Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med*, 169(4), 384-390. doi:10.1001/archinternmed.2008.560
- Giron-Prieto, M. S., Del Carmen Cano-Garcia, M., Arrabal-Polo, M. A., Poyatos-Andujar, A., Quesada-Charneco, M., de Haro-Munoz, T., . . . Arrabal-Martin, M. (2016). Analysis of vitamin D deficiency in calcium stone-forming patients. *Int Urol Nephrol*. doi:10.1007/s11255-016-1290-3
- Glasziou, P. P., Irwig, L., Heritier, S., Simes, R. J., & Tonkin, A. (2008). Monitoring cholesterol levels: measurement error or true change? *Ann Intern Med*, 148(9), 656-661.

- Glendenning, P. (2015). Measuring vitamin D. *Aust Prescr*, 38(1), 12-15.
- Glueck, C. J., Jetty, V., Rothschild, M., Duhon, G., Shah, P., Prince, M., . . . Goldenberg, N. (2016). Associations between serum 25-hydroxyvitamin D and lipids, lipoprotein cholesterol, and homocysteine. *North Am J Med Sci*, 8(7), 284.
- Goldfarb, D. S. (2012). A woman with recurrent calcium phosphate kidney stones. *Clin J Am Soc Nephrol*, 7(7), 1172-1178.
- Gombart, A. F., Borregaard, N., & Koeffler, H. P. (2005). Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *Faseb j*, 19(9), 1067-1077. doi:10.1096/fj.04-3284com
- Goncalves, A., Gleize, B., Bott, R., Nowicki, M., Amiot, M. J., Lairon, D., . . . Reboul, E. (2011). Phytosterols can impair vitamin D intestinal absorption in vitro and in mice. *Mol Nutr Food Res*, 55 Suppl 2, S303-311. doi:10.1002/mnfr.201100055
- Goncharov, N. V., Avdonin, P. V., Nadeev, A. D., Zharkikh, I. L., & Jenkins, R. O. (2015). Reactive oxygen species in pathogenesis of atherosclerosis. *Curr Pharm Des*, 21(9), 1134-1146.
- Gonzalez-Molero, I., Rojo-Martinez, G., Morcillo, S., Gutierrez, C., Rubio, E., Perez-Valero, V., . . . Soriguer, F. (2013). Hypovitaminosis D and incidence of obesity: a prospective study. *Eur J Clin Nutr*, 67(6), 680-682. doi:10.1038/ejcn.2013.48
- Gonzalez, E. A., Sachdeva, A., Oliver, D. A., & Martin, K. J. (2004). Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. *Am J Nephrol*, 24(5), 503-510. doi:10.1159/000081023
- Gonzalez, F. J., & Moschetta, A. (2014). Potential role of the vitamin D receptor in control of cholesterol levels. *Gastroenterology*, 146(4), 899-902. doi:10.1053/j.gastro.2014.02.022
- Gower, L. B., Amos, F. F., & Khan, S. R. (2010). Mineralogical signatures of stone formation mechanisms. *Urol Res*, 38(4), 281-292. doi:10.1007/s00240-010-0288-z
- Gozdzik, A., Zhu, J., Wong, B. Y.-L., Fu, L., Cole, D. E., & Parra, E. J. (2011). Association of vitamin D binding protein (VDBP) polymorphisms and serum

- 25 (OH) D concentrations in a sample of young Canadian adults of different ancestry. *J Steroid Biochem Mol Biol*, 127(3), 405-412.
- Granata, S., Zaza, G., Simone, S., Villani, G., Latorre, D., Pontrelli, P., . . . Pertosa, G. (2009). Mitochondrial dysregulation and oxidative stress in patients with chronic kidney disease. *BMC Genomics*, 10, 388. doi:10.1186/1471-2164-10-388
- Grandi, N. C., Breitling, L. P., & Brenner, H. (2010). Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. *Prev Med*, 51(3-4), 228-233. doi:10.1016/j.ypmed.2010.06.013
- Grossmann, R. E., & Tangpricha, V. (2010). Evaluation of vehicle substances on vitamin D bioavailability: a systematic review. *Mol Nutr Food Res*, 54(8), 1055-1061. doi:10.1002/mnfr.200900578
- Grover, P. K., & Ryall, R. L. (2002). Effect of prothrombin and its activation fragments on calcium oxalate crystal growth and aggregation in undiluted human urine in vitro: relationship between protein structure and inhibitory activity. *Clin Sci (Lond)*, 102(4), 425-434.
- Grover, P. K., Ryall, R. L., & Marshall, V. R. (1990). Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallization in human urine? *Clin Chim Acta*, 190(3), 223-238.
- Gu, J.-m., Xiao, W.-j., He, J.-w., Zhang, H., Hu, W.-w., Hu, Y.-q., . . . Yu, J.-b. (2009). Association between VDR and ESR1 gene polymorphisms with bone and obesity phenotypes in Chinese male nuclear families. *Acta Pharmacologica Sinica*, 30(12), 1634-1642.
- Guijarro, C., & Egido, J. (2001). Transcription factor-kappa B (NF-kappa B) and renal disease. *Kidney Int*, 59(2), 415-424. doi:10.1046/j.1523-1755.2001.059002415.x
- Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G., & Boissier, M. C. (2010). Vitamin D and inflammation. *Joint Bone Spine*, 77(6), 552-557. doi:10.1016/j.jbspin.2010.09.018
- Ha, H., Oh, E. Y., & Lee, H. B. (2009). The role of plasminogen activator inhibitor 1 in renal and cardiovascular diseases. *Nat Rev Nephrol*, 5(4), 203-211.
- Haas, M. J., Jafri, M., Wehmeier, K. R., Onstead-Haas, L. M., & Mooradian, A. D. (2016). Inhibition of endoplasmic reticulum stress and oxidative stress by

- vitamin D in endothelial cells. *Free Radic Biol Med*, 99, 1-10. doi:10.1016/j.freeradbiomed.2016.07.020
- Haddad, J. G., Matsuoka, L. Y., Hollis, B. W., Hu, Y. Z., & Wortsman, J. (1993). Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest*, 91(6), 2552-2555. doi:10.1172/jci116492
- Haffner, S. M., Kennedy, E., Gonzalez, C., Stern, M. P., & Miettinen, H. (1996). A prospective analysis of the HOMA model. The Mexico City Diabetes Study. *Diabetes Care*, 19(10), 1138-1141.
- Hagenau, T., Vest, R., Gissel, T. N., Poulsen, C. S., Erlandsen, M., Mosekilde, L., & Vestergaard, P. (2009). Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. *Osteoporos Int*, 20(1), 133-140. doi:10.1007/s00198-008-0626-y
- Hagstrom, E., Hellman, P., Larsson, T. E., Ingelsson, E., Berglund, L., Sundstrom, J., . . . Arnlov, J. (2009). Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation*, 119(21), 2765-2771. doi:10.1161/circulationaha.108.808733
- Hamano, S., Nakatsu, H., Suzuki, N., Tomioka, S., Tanaka, M., & Murakami, S. (2005). Kidney stone disease and risk factors for coronary heart disease. *Intl J Urol*, 12(10), 859-863.
- Han, H., Segal, A. M., Seifter, J. L., & Dwyer, J. T. (2015). Nutritional Management of Kidney Stones (Nephrolithiasis). *Clin Nutr Res*, 4(3), 137-152. doi:10.7762/cnr.2015.4.3.137
- Han, H. J., Lim, M. J., Lee, Y. J., Lee, J. H., Yang, I. S., & Taub, M. (2007). Uric acid inhibits renal proximal tubule cell proliferation via at least two signaling pathways involving PKC, MAPK, cPLA2, and NF-kappaB. *Am J Physiol Renal Physiol*, 292(1), F373-381. doi:10.1152/ajprenal.00104.2006
- Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*, 352(16), 1685-1695. doi:10.1056/NEJMr043430
- Hansson, G. K., & Hermansson, A. (2011). The immune system in atherosclerosis. *Nature immunology*, 12(3), 204-212.
- Harja, E., Bu, D. X., Hudson, B. I., Chang, J. S., Shen, X., Hallam, K., . . . Schmidt, A. M. (2008). Vascular and inflammatory stresses mediate atherosclerosis via RAGE and its ligands in apoE^{-/-} mice. *J Clin Invest*, 118(1), 183-194. doi:10.1172/jci32703

- Harris, R. A., Pedersen-White, J., Guo, D. H., Stallmann-Jorgensen, I. S., Keeton, D., Huang, Y., . . . Dong, Y. (2011). Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens*, 24(5), 557-562. doi:10.1038/ajh.2011.12
- Harris, S. S., & Dawson-Hughes, B. (1998). Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr*, 67(6), 1232-1236.
- Harris, S. S., & Dawson-Hughes, B. (2002). Plasma vitamin D and 25OHD responses of young and old men to supplementation with vitamin D₃. *J Am Coll Nutr*, 21(4), 357-362.
- Hart, G. R., Furniss, J. L., Laurie, D., & Durham, S. K. (2005). Measurement of vitamin D status: background, clinical use, and methodologies. *J Clin Lab*, 52(7-8), 335-343.
- Harvey, N., Dennison, E., & Cooper, C. (2014). Osteoporosis: a lifecourse approach. *J Bone Miner Res*, 29(9), 1917-1925. doi:10.1002/jbmr.2286
- Hasegawa, H., Nagano, N., Urakawa, I., Yamazaki, Y., Iijima, K., Fujita, T., . . . Shimada, T. (2010). Direct evidence for a causative role of FGF23 in the abnormal renal phosphate handling and vitamin D metabolism in rats with early-stage chronic kidney disease. *Kidney Int*, 78(10), 975-980. doi:10.1038/ki.2010.313
- Hasna, A., Meiyappan, K., Periyasam, S. G., Kalyaperumal, M., Bobby, Z., & Subramaniam, A. (2015). Is urolithiasis associated with increased levels of high sensitivity C-reactive protein and interleukin-6 in diabetic patients? *J Clin Diagn Res: JCDR*, 9(3), BC01-03.
- Hathcock, J. N., Shao, A., Vieth, R., & Heaney, R. (2007). Risk assessment for vitamin D. *Am J Clin Nutr*, 85(1), 6-18.
- Haussler, M. R., Jurutka, P. W., Mizwicki, M., & Norman, A. W. (2011). Vitamin D receptor (VDR)-mediated actions of 1alpha,25(OH)(2)vitamin D(3): genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab*, 25(4), 543-559. doi:10.1016/j.beem.2011.05.010
- Hayes, C. E., Nashold, F. E., Spach, K. M., & Pedersen, L. B. (2003). The immunological functions of the vitamin D endocrine system. *Cell Mol Biol (Noisy-le-grand)*, 49(2), 277-300.

- Health Council of the Netherlands. (2012). Evaluation of dietary reference values for vitamin D. *The Hague: Health Council of the Netherlands*.
- Heaney, R. P. (2008). Vitamin D in health and disease. *Clin J Am Soc Nephrol*, 3(5), 1535-1541. doi:10.2215/CJN.01160308
- Heaney, R. P. (2008). Vitamin D: criteria for safety and efficacy. *Nutr Rev*, 66(10 Suppl 2), S178-181. doi:10.1111/j.1753-4887.2008.00102.x
- Heaney, R. P. (2012). Vitamin D—baseline status and effective dose. *N Engl J Med*, 367(1), 77-78.
- Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F., & Barger-Lux, M. J. (2003). Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr*, 77(1), 204-210.
- Heaney, R. P., Recker, R. R., Grote, J., Horst, R. L., & Armas, L. A. (2011). Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab*, 96(3), E447-452. doi:10.1210/jc.2010-2230
- Helidan, A., Kosonen, M., & Tapanainen, H. (2013). The National FINDIET 2012 Survey Helsinki National Institute for Health and Welfare 2013 Report No 16/2013. Available at: <https://www.helsinki.fi/en/health-and-welfare>
- Helvig, C. F., Cuerrier, D., Hosfield, C. M., Ireland, B., Kharebov, A. Z., Kim, J. W., . . . Petkovich, M. (2010). Dysregulation of renal vitamin D metabolism in the uremic rat. *Kidney Int*, 78(5), 463-472. doi:10.1038/ki.2010.168
- Henry, H. L. (2011). Regulation of vitamin D metabolism. *Best Pract Res Clin Endocrinol Metab*, 25(4), 531-541. doi:10.1016/j.beem.2011.05.003
- Herman-Edelstein, M., Scherzer, P., Tobar, A., Levi, M., & Gaftor, U. (2014). Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy. *J Lipid Res*, 55(3), 561-572. doi:10.1194/jlr.P040501
- Heshmat, R., Tabatabaei-Malazy, O., Abbaszadeh-Ahranjani, S., Shahbazi, S., Khooshehchin, G., Bandarian, F., & Larijani, B. (2012). Effect of vitamin D on insulin resistance and anthropometric parameters in Type 2 diabetes; a randomized double-blind clinical trial. *Daru*, 20(1), 10. doi:10.1186/2008-2231-20-10
- Hess, B. (1991). The role of Tamm-Horsfall glycoprotein and Nephrocalcin in calcium oxalate monohydrate crystallization processes. *Scanning Microsc*, 5(3), 689-695; discussion 696.

- Hilger, J., Friedel, A., Herr, R., Rausch, T., Roos, F., Wahl, D. A., . . . Hoffmann, K. (2014). A systematic review of vitamin D status in populations worldwide. *Br J Nutr*, *111*(1), 23-45. doi:10.1017/s0007114513001840
- Hill, J. (2005). Adherence with drug therapy in the rheumatic diseases Part two: measuring and improving adherence. *Musculoskeletal Care*, *3*(3), 143-156.
- Hoenderop, J. G., Dardenne, O., Van Abel, M., Van Der Kemp, A. W., Van Os, C. H., St -Arnaud, R., & Bindels, R. J. (2002). Modulation of renal Ca²⁺ transport protein genes by dietary Ca²⁺ and 1,25-dihydroxyvitamin D₃ in 25-hydroxyvitamin D₃-1alpha-hydroxylase knockout mice. *Faseb j*, *16*(11), 1398-1406. doi:10.1096/fj.02-0225com
- Hoenderop, J. G., van Leeuwen, J. P., van der Eerden, B. C., Kersten, F. F., van der Kemp, A. W., Merillat, A. M., . . . Bindels, R. J. (2003). Renal Ca²⁺ wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. *J Clin Invest*, *112*(12), 1906-1914. doi:10.1172/jci19826
- Hohn, A., Defosse, J., Becker, S., Steffen, C., Wappler, F., & Sakka, S. (2013). Non-invasive continuous arterial pressure monitoring with Nexfin® does not sufficiently replace invasive measurements in critically ill patients. *Br J Anaesth*, *111*(2), 178-184.
- Holick, M. F. (1994). McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. *Am J Clin Nutr*, *60*(4), 619-630.
- Holick, M. F. (1996). Vitamin D and bone health. *J Nutr*, *126*(4S), 1159S.
- Holick, M. F. (2004). Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr*, *80*(6 Suppl), 1678S-1688S.
- Holick, M. F. (2005). The vitamin D epidemic and its health consequences. *J Nutr*, *135*(11), 2739S-2748S.
- Holick, M. F. (2006). High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*, *81*(3), 353-373. doi:10.4065/81.3.353
- Holick, M. F. (2007). Vitamin D deficiency. *N Engl J Med*, *357*(3), 266-281. doi:10.1056/NEJMra070553
- Holick, M. F. (2010). Vitamin D: extraskeletal health. *Endocrinol Metab Clin North Am*, *39*(2), 381-400, table of contents. doi:10.1016/j.ecl.2010.02.016
- Holick, M. F. (2011). Vitamin D: evolutionary, physiological and health perspectives. *Curr Drug Targets*, *12*(1), 4-18.

- Holick, M. F., Biancuzzo, R. M., Chen, T. C., Klein, E. K., Young, A., Bibuld, D., . . . Tannenbaum, A. D. (2008). Vitamin D₂ is as effective as vitamin D₃ in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab*, 93(3), 677-681. doi:10.1210/jc.2007-2308
- Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., . . . Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, 96(7), 1911-1930.
- Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., . . . Endocrine, S. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, 96(7), 1911-1930. doi:10.1210/jc.2011-0385
- Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*, 87(4), 1080S-1086S.
- Holick, M. F., Chen, T. C., Lu, Z., & Sauter, E. (2007). Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res*, 22 Suppl 2, V28-33. doi:10.1359/jbmr.07s211
- Holick, M. F., MacLaughlin, J. A., & Doppelt, S. H. (1981). Regulation of cutaneous previtamin D₃ photosynthesis in man: skin pigment is not an essential regulator. *Science*, 211(4482), 590-593.
- Hollis, B. W., Johnson, D., Hulsey, T. C., Ebeling, M., & Wagner, C. L. (2011). Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*, 26(10), 2341-2357. doi:10.1002/jbmr.463
- Hollis, B. W., & Wagner, C. L. (2004). Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr*, 80(6 Suppl), 1752S-1758S.
- Holzer, M., Birner-Gruenberger, R., Stojakovic, T., El-Gamal, D., Binder, V., Wadsack, C., . . . Marsche, G. (2011). Uremia alters HDL composition and function. *J Am Soc Nephrol*, 22(9), 1631-1641. doi:10.1681/ASN.2010111144

- Hopkins, K., Lehmann, E., & Gosling, R. (1994). Aortic compliance measurements: a non-invasive indicator of atherosclerosis? *The Lancet*, 343(8911), 1447.
- Horvath, I. G., Nemeth, A., Lenkey, Z., Alessandri, N., Tufano, F., Kis, P., . . . Cziraki, A. (2010). Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. *J Hypertens*, 28(10), 2068-2075. doi:10.1097/HJH.0b013e32833c8a1a
- Hosseini-nezhad, A., & Holick, M. F. (2013). Vitamin D for health: a global perspective. *Mayo Clin Proc*, 88(7), 720-755. doi:10.1016/j.mayocp.2013.05.011
- Houghton, L. A., & Vieth, R. (2006). The case against ergocalciferol (vitamin D₂) as a vitamin supplement. *Am J Clin Nutr*, 84(4), 694-697.
- Hoyland, A., Dye, L., & Lawton, C. L. (2009). A systematic review of the effect of breakfast on the cognitive performance of children and adolescents. *Nutr Res Rev*, 22(02), 220-243.
- Hsi, R. S., Spieker, A. J., Stoller, M. L., Jacobs, D. R., Jr., Reiner, A. P., McClelland, R. L., . . . Sorensen, M. D. (2016). Coronary Artery Calcium Score and Association with Recurrent Nephrolithiasis: The Multi-Ethnic Study of Atherosclerosis. *J Urol*, 195(4), 971-976.
- Hu, M. C., Shiizaki, K., Kuro-o, M., & Moe, O. W. (2013). Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol*, 75, 503-533. doi:10.1146/annurev-physiol-030212-183727
- Hu, P., Hu, B., Wang, J., Lu, L., & Qin, Y. H. (2011). Modulation of vitamin D signaling is a potential therapeutic target to lower cardiovascular risk in chronic kidney disease. *Med Sci Monit*, 17(6), Hy14-20.
- Hu, P., Lu, L., Hu, B., & Du, P. F. (2009). Characteristics of lipid metabolism under different urinary protein excretion in children with primary nephrotic syndrome. *Scandinavian J Clin Lab Invest*, 69(6), 680-686.
- Huang, H.-S., Ma, M.-C., Chen, C.-F., & Chen, J. (2003). Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. *Urology*, 62(6), 1123-1128.
- Huhtakangas, J. A., Olivera, C. J., Bishop, J. E., Zanello, L. P., & Norman, A. W. (2004). The vitamin D receptor is present in caveolae-enriched plasma

- membranes and binds 1 alpha,25(OH)₂-vitamin D₃ in vivo and in vitro. *Mol Endocrinol*, 18(11), 2660-2671. doi:10.1210/me.2004-0116
- Hur, S. J., Kim, D. M., Lim, K. H., Yoon, S. H., Chung, H. C., Lee, J. S., & Park, J. (2009). Vitamin D levels and their relationship with cardiac biomarkers in chronic hemodialysis patients. *J Korean Med Sci*, 24 Suppl, S109-114. doi:10.3346/jkms.2009.24.S1.S109
- Hypponen, E., & Power, C. (2007). Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr*, 85(3), 860-868.
- Inci, M., Demirtas, A., Sarli, B., Akinsal, E., & Baydilli, N. (2012). Association between body mass index, lipid profiles, and types of urinary stones. *Ren Fail*, 34(9), 1140-1143. doi:10.3109/0886022X.2012.713298
- Issa, L. L., Leong, G. M., Sutherland, R. L., & Eisman, J. A. (2002). Vitamin D analogue-specific recruitment of vitamin D receptor coactivators. *J Bone Miner Res*, 17(5), 879-890. doi:10.1359/jbmr.2002.17.5.879
- Istvan, E. S., & Deisenhofer, J. (2001). Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*, 292(5519), 1160-1164. doi:10.1126/science.1059344
- Ito, S., Osaka, M., Higuchi, Y., Nishijima, F., Ishii, H., & Yoshida, M. (2010). Indoxyl sulfate induces leukocyte-endothelial interactions through up-regulation of E-selectin. *J Biol Chem*, 285(50), 38869-38875. doi:10.1074/jbc.M110.166686
- Jabbar, Z., Aggarwal, P. K., Chandel, N., Kohli, H. S., Gupta, K. L., Sakhuja, V., & Jha, V. (2009). High prevalence of vitamin D deficiency in north Indian adults is exacerbated in those with chronic kidney disease. *Nephrology*, 14(3), 345-349.
- Jablonski, K. L., Chonchol, M., Pierce, G. L., Walker, A. E., & Seals, D. R. (2011). 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension*, 57(1), 63-69. doi:10.1161/HYPERTENSIONAHA.110.160929
- Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *J Hum Evol*, 39(1), 57-106. doi:10.1006/jhev.2000.0403
- James, W. P. (2008). The epidemiology of obesity: the size of the problem. *J Intern Med*, 263(4), 336-352. doi:10.1111/j.1365-2796.2008.01922.x

- Jamka, M., Woźniewicz, M., Jeszka, J., Mardas, M., Bogdański, P., & Stelmach-Mardas, M. (2015). The effect of vitamin D supplementation on insulin and glucose metabolism in overweight and obese individuals: systematic review with meta-analysis. *Scientific reports*, 5.
- Jamka, M., Woźniewicz, M., Walkowiak, J., Bogdański, P., Jeszka, J., & Stelmach-Mardas, M. (2015). The effect of vitamin D supplementation on selected inflammatory biomarkers in obese and overweight subjects: a systematic review with meta-analysis. *Eur J Nutr*, 1-14.
- Japelt, R. B., & Jakobsen, J. (2013). Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. *Front Plant Sci*, 4, 136. doi:10.3389/fpls.2013.00136
- Jastrzebski, Z., Kortas, J., Kaczor, K., & Antosiewicz, J. (2016). Vitamin D Supplementation Causes a Decrease in Blood Cholesterol in Professional Rowers. *J Nutr Sci Vitaminol (Tokyo)*, 62(2), 88-92. doi:10.3177/jnsv.62.88
- Jatoi, N. A., Mahmud, A., Bennett, K., & Feely, J. (2009). Assessment of arterial stiffness in hypertension: comparison of oscillometric (Arteriograph), piezoelectronic (Colson) and tonometric (Sphygmocor) techniques. *J Hypertens*, 27(11), 2186-2191.
- Javadi, M. K., Crozier, S. R., Harvey, N. C., Gale, C. R., Dennison, E. M., Boucher, B. J., . . . Princess Anne Hospital Study, G. (2006). Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet*, 367(9504), 36-43. doi:10.1016/S0140-6736(06)67922-1
- Javed, A., Vella, A., Balagopal, P. B., Fischer, P. R., Weaver, A. L., Piccinini, F., . . . Kumar, S. (2015). Cholecalciferol supplementation does not influence beta-cell function and insulin action in obese adolescents: a prospective double-blind randomized trial. *J Nutr*, 145(2), 284-290. doi:10.3945/jn.114.202010
- Jha, P., Dolan, L. M., Khoury, P. R., Urbina, E. M., Kimball, T. R., & Shah, A. S. (2015). Low Serum Vitamin D Levels Are Associated With Increased Arterial Stiffness in Youth With Type 2 Diabetes. *Diabetes Care*, 38(8), 1551-1557.
- Johansson, L., Solvoll, K., Bjørneboe, G.-E., & Drevon, C. A. (1998). Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *Am J Clin Nutr*, 68(2), 266-274.

- Jonassen, J. A., Kohjimoto, Y., Scheid, C. R., & Schmidt, M. (2005). Oxalate toxicity in renal cells. *Urological research*, 33(5), 329-339.
- Jones, G. (2008). Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr*, 88(2), 582S-586S.
- Jones, G., Prosser, D. E., & Kaufmann, M. (2012). 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys*, 523(1), 9-18. doi:10.1016/j.abb.2011.11.003
- Jorde, R., Figenschau, Y., Hutchinson, M., Emaus, N., & Grimnes, G. (2010). High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile. *Eur J Clin Nutr*, 64(12), 1457-1464.
- Jorde, R., & Grimnes, G. (2011). Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res*, 50(4), 303-312. doi:10.1016/j.plipres.2011.05.001
- Joshi, S., Peck, A. B., & Khan, S. R. (2013). NADPH oxidase as a therapeutic target for oxalate induced injury in kidneys. *Oxid Med Cell Longev*, 2013, 462361. doi:10.1155/2013/462361
- Ju, S. Y., Jeong, H. S., & Kim, D. H. (2013). Blood vitamin D status and metabolic syndrome in the general adult population: a dose-response meta-analysis. *The J Clin Endocrinol Metab*, 99(3), 1053-1063.
- Jungert, A., Roth, H. J., & Neuhauser-Berthold, M. (2012). Serum 25-hydroxyvitamin D3, parathyroid hormone and blood pressure in an elderly cohort from Germany: a cross-sectional study. *Nutr Metab (Lond)*, 9(1), 20. doi:10.1186/1743-7075-9-20
- Kahn, B. B., & Flier, J. S. (2000). Obesity and insulin resistance. *J Clin Invest*, 106(4), 473-481.
- Kamen, D., & Aranow, C. (2008). Vitamin D in systemic lupus erythematosus. *Curr Opin Rheumatol*, 20(5), 532-537. doi:10.1097/BOR.0b013e32830a991b
- Kamen, D., Barron, M., Hollis, B., Oates, J., Bouali, H., Bruner, G., . . . Gilkeson, G. (2005). *Correlation of vitamin D deficiency with lupus disease measures among Sea Island Gullah African Americans*. Paper presented at the ARTHRITIS AND RHEUMATISM.
- Kampmann, U., Mosekilde, L., Juhl, C., Moller, N., Christensen, B., Rejnmark, L., . . . Orskov, L. (2014). Effects of 12 weeks high dose vitamin D3 treatment on insulin sensitivity, beta cell function, and metabolic markers in patients with

- type 2 diabetes and vitamin D insufficiency - a double-blind, randomized, placebo-controlled trial. *Metabolism*, 63(9), 1115-1124. doi:10.1016/j.metabol.2014.06.008
- Kamycheva, E., Jorde, R., Figenschau, Y., & Haug, E. (2007). Insulin sensitivity in subjects with secondary hyperparathyroidism and the effect of a low serum 25-hydroxyvitamin D level on insulin sensitivity. *J Endocrinol Invest*, 30(2), 126-132. doi:10.1007/bf03347410
- Kandula, P., Dobre, M., Schold, J. D., Schreiber, M. J., Mehrotra, R., & Navaneethan, S. D. (2011). Vitamin D supplementation in chronic kidney disease: a systematic review and meta-analysis of observational studies and randomized controlled trials. *Clin J Am Soc Nephrol*, 6(1), 50-62.
- Kaneko, K., Kobayashi, R., Yasuda, M., Izumi, Y., Yamanobe, T., & Shimizu, T. (2012). Comparison of matrix proteins in different types of urinary stone by proteomic analysis using liquid chromatography-tandem mass spectrometry. *Int J Urol*, 19(8), 765-772. doi:10.1111/j.1442-2042.2012.03005.x
- Kang, H. W., Lee, S. K., Kim, W. T., Kim, Y. J., Yun, S. J., Lee, S. C., & Kim, W. J. (2014). Hypertriglyceridemia and low high-density lipoprotein cholesterol are associated with increased hazard for urolithiasis. *J Endourol*, 28(8), 1001-1005. doi:10.1089/end.2014.0135
- Karohl, C., Su, S., Kumari, M., Tangpricha, V., Veledar, E., Vaccarino, V., & Raggi, P. (2010). Heritability and seasonal variability of vitamin D concentrations in male twins. *Am J Clin Nutr*, 92(6), 1393-1398. doi:10.3945/ajcn.2010.30176
- Kassi, E., Adamopoulos, C., Basdra, E. K., & Papavassiliou, A. G. (2013). Role of vitamin D in atherosclerosis. *Circulation*, 128(23), 2517-2531. doi:10.1161/circulationaha.113.002654
- Kassi, E., Pervanidou, P., Kaltsas, G., & Chrousos, G. (2011). Metabolic syndrome: definitions and controversies. *BMC medicine*, 9(1), 1.
- Keddis, M. T., & Rule, A. D. (2013). Nephrolithiasis and loss of kidney function. *Curr Opin Nephrol Hypertens*, 22(4), 390-396. doi:10.1097/MNH.0b013e32836214b9
- Kelishadi, R., Farajzadegan, Z., & Bahreynian, M. (2014). Association between vitamin D status and lipid profile in children and adolescents: a systematic review and meta-analysis. *Int J Food Sci Nutr*, 65(4), 404-410.

- Khajehdehi, P. (2000). Effect of vitamins on the lipid profile of patients on regular hemodialysis. *Scand J Urol Nephrol*, 34(1), 62-66.
- Khan, M. I., Bielecka, Z. F., Najm, M. Z., Bartnik, E., Czarnecki, J. S., Czarnecka, A. M., & Szczyluk, C. (2014). Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches (review). *Int J Oncol*, 44(2), 349-363. doi:10.3892/ijo.2013.2204
- Khan, S. R. (2014). Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. *Transl Androl Urol*, 3(3), 256.
- Khan, S. R., Byer, K. J., Thamilselvan, S., Hackett, R. L., McCormack, W. T., Benson, N. A., . . . Erdos, G. W. (1999). Crystal-cell interaction and apoptosis in oxalate-associated injury of renal epithelial cells. *J Am Soc Nephrol*, 10 Suppl 14, S457-463.
- Khan, S. R., Glenton, P. A., & Byer, K. J. (2006). Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. *Kidney Int*, 70(5), 914-923. doi:10.1038/sj.ki.5001699
- Khan, S. R., & Kok, D. J. (2004). Modulators of urinary stone formation. *Front Biosci*, 9(629), 1450-1482.
- Khodarahmi, M., & Azadbakht, L. (2014). The association between different kinds of fat intake and breast cancer risk in women. *Int J Prev Med*, 5(1), 6-15.
- Kienreich, K., Grübler, M., Tomaschitz, A., Schmid, J., Verheyen, N., Rutters, F., . . . Pilz, S. (2013). Vitamin D, arterial hypertension & cerebrovascular disease. *Indian J Med Res*, 137(4), 669.
- Kienreich, K., Tomaschitz, A., Verheyen, N., Pieber, T., Gaksch, M., Grübler, M. R., & Pilz, S. (2013). Vitamin D and cardiovascular disease. *Nutrients*, 5(8), 3005-3021.
- Kim, C. S., & Kim, S. W. (2014). Vitamin D and chronic kidney disease. *The Korean J Intern Med*, 29(4), 416-427.
- Kim, J.-a., Montagnani, M., Chandrasekran, S., & Quon, M. J. (2012). Role of lipotoxicity in endothelial dysfunction. *Heart failure clinics*, 8(4), 589-607.
- Kim, S., Yamazaki, M., Zella, L. A., Shevde, N. K., & Pike, J. W. (2006). Activation of receptor activator of NF- κ B ligand gene expression by 1, 25-dihydroxyvitamin D₃ is mediated through multiple long-range enhancers. *Mol Cell Biol*, 26(17), 6469-6486.

- Kitanaka, S., Takeyama, K., Murayama, A., Sato, T., Okumura, K., Nogami, M., . . . Kato, S. (1998). Inactivating mutations in the 25-hydroxyvitamin D3 1 α -hydroxylase gene in patients with pseudovitamin D-deficiency rickets. *N Engl J Med*, 338(10), 653-661. doi:10.1056/NEJM199803053381004
- Kleinman, J. G., Wesson, J. A., & Hughes, J. (2004). Osteopontin and calcium stone formation. *Nephron Physiol*, 98(2), p43-47. doi:10.1159/000080263
- Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*, 5(4), 1218-1240.
- Koba, S., & Hirano, T. (2011). [Dyslipidemia and atherosclerosis]. *Nihon rinsho. Japanese J Clin Med*, 69(1), 138-143.
- Kong, J., & Li, Y. C. (2006). Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. *Am J Physiol Endocrinol Metab*, 290(5), E916-924. doi:10.1152/ajpendo.00410.2005
- Konova, E., Baydanoff, S., Atanasova, M., & Velkova, A. (2004). Age-related changes in the glycation of human aortic elastin. *Exp Gerontol*, 39(2), 249-254. doi:10.1016/j.exger.2003.10.003
- Koszewski, N. J., Alimov, A. P., Park-Sarge, O. K., & Malluche, H. H. (2004). Suppression of the human parathyroid hormone promoter by vitamin D involves displacement of NF-Y binding to the vitamin D response element. *J Biol Chem*, 279(41), 42431-42437. doi:10.1074/jbc.M407742200
- Kota, S. K., Kota, S. K., Jammula, S., Meher, L. K., Panda, S., Tripathy, P. R., & Modi, K. D. (2011). Renin-angiotensin system activity in vitamin D deficient, obese individuals with hypertension: An urban Indian study. *Indian J Endocrinol Metab*, 15(8), 395.
- Krivosikova, Z., Gajdos, M., & Sebekova, K. (2015). Vitamin D Levels Decline with Rising Number of Cardiometabolic Risk Factors in Healthy Adults: Association with Adipokines, Inflammation, Oxidative Stress and Advanced Glycation Markers. *PLoS One*, 10(6), e0131753. doi:10.1371/journal.pone.0131753
- Kruger, I. M., Kruger, M. C., Doak, C. M., Schutte, A. E., Huisman, H. W., Van Rooyen, J. M., . . . Fourie, C. M. (2013). The association of 25 (OH) D with blood pressure, pulse pressure and carotid-radial pulse wave velocity in African women. *PLoS One*, 8(1), e54554.

- Kumar, V., Farell, G., & Lieske, J. C. (2003). Whole urinary proteins coat calcium oxalate monohydrate crystals to greatly decrease their adhesion to renal cells. *J Urol*, 170(1), 221-225. doi:10.1097/01.ju.0000059540.36463.9f
- Kunutsor, S. K., Apekey, T. A., & Steur, M. (2013). Vitamin D and risk of future hypertension: meta-analysis of 283,537 participants. *Eur J Epidemiol*, 28(3), 205-221.
- Kunutsor, S. K., Burgess, S., Munroe, P. B., & Khan, H. (2014). Vitamin D and high blood pressure: causal association or epiphenomenon? *Eur J Epidemiol*, 29(1), 1-14. doi:10.1007/s10654-013-9874-z
- Kurutz, J. W., Carvalho, M., & Nakagawa, Y. (2003). Nephrocalcin isoforms coat crystal surfaces and differentially affect calcium oxalate monohydrate crystal morphology, growth, and aggregation. *J Cryst Growth*, 255(3), 392-402.
- Kuzuya, M., Asai, T., Kanda, S., Maeda, K., Cheng, X., & Iguchi, A. (2001). Glycation cross-links inhibit matrix metalloproteinase-2 activation in vascular smooth muscle cells cultured on collagen lattice. *Diabetologia*, 44(4), 433-436.
- Ladizesky, M., Lu, Z., Oliveri, B., San Roman, N., Diaz, S., Holick, M. F., & Mautalen, C. (1995). Solar ultraviolet B radiation and photoproduction of vitamin D3 in central and southern areas of Argentina. *J Bone Miner Res*, 10(4), 545-549. doi:10.1002/jbmr.5650100406
- Lahoute, C., Herbin, O., Mallat, Z., & Tedgui, A. (2011). Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat Rev Cardiol*, 8(6), 348-358. doi:10.1038/nrcardio.2011.62
- Lai, Y. H., & Fang, T. C. (2013). The pleiotropic effect of vitamin d. *ISRN Nephrol*, 2013, 898125. doi:10.5402/2013/898125
- Lakatta, E. G., & Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises part I: aging arteries: a “set up” for vascular disease. *Circulation*, 107(1), 139-146.
- Lamb, J., Ramaswamy, S., Ford, H. L., Contreras, B., Martinez, R. V., Kittrell, F. S., . . . Ewen, M. E. (2003). A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. *Cell*, 114(3), 323-334.
- Lamberg-Allardt, C., Brustad, M., Meyer, H. E., & Steingrimsdottir, L. (2013). Vitamin D - a systematic literature review for the 5th edition of the Nordic

- Lantelme, P., Mestre, C., Lievre, M., Gressard, A., & Milon, H. (2002). Heart rate an important confounder of pulse wave velocity assessment. *Hypertension*, 39(6), 1083-1087.
- Lark, R. K., Lester, G. E., Ontjes, D. A., Blackwood, A. D., Hollis, B. W., Hensler, M. M., & Aris, R. M. (2001). Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients. *Am J Clin Nutr*, 73(3), 602-606.
- Lassegue, B., Sorescu, D., Szocs, K., Yin, Q., Akers, M., Zhang, Y., . . . Griendling, K. K. (2001). Novel gp91(phox) homologues in vascular smooth muscle cells : nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. *Circ Res*, 88(9), 888-894.
- Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., . . . Struijker-Boudier, H. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*, 27(21), 2588-2605.
- Lawson, M., & Thomas, M. (1999). Vitamin D concentrations in Asian children aged 2 years living in England: population survey. *BMJ*, 318(7175), 28.
- Lee, J. H., O'Keefe, J. H., Bell, D., Hensrud, D. D., & Holick, M. F. (2008). Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol*, 52(24), 1949-1956. doi:10.1016/j.jacc.2008.08.050
- Lee, P., Greenfield, J. R., Seibel, M. J., Eisman, J. A., & Center, J. R. (2009). Adequacy of vitamin D replacement in severe deficiency is dependent on body mass index. *Am J Med*, 122(11), 1056-1060. doi:10.1016/j.amjmed.2009.06.008
- Lee, Y. T., Ng, H. Y., Kuo, C. C., Chen, T. C., Wu, C. S., Chiu, T. T., . . . Lee, C. T. (2013). Comparison between calcitriol and calcitriol plus low-dose cinacalcet for the treatment of moderate to severe secondary hyperparathyroidism in chronic dialysis patients. *Nutrients*, 5(4), 1336-1348. doi:10.3390/nu5041336
- Lehmann, B., & Meurer, M. (2010). Vitamin D metabolism. *Dermatol Ther*, 23(1), 2-12. doi:10.1111/j.1529-8019.2009.01286.x

- Lehmann, U., Hirche, F., Stangl, G. I., Hinz, K., Westphal, S., & Dierkes, J. (2013). Bioavailability of vitamin D(2) and D(3) in healthy volunteers, a randomized placebo-controlled trial. *J Clin Endocrinol Metab*, 98(11), 4339-4345. doi:10.1210/jc.2012-4287
- Leichtmann, G. A., Bengoa, J. M., Bolt, M. J., & Sitrin, M. D. (1991). Intestinal absorption of cholecalciferol and 25-hydroxycholecalciferol in patients with both Crohn's disease and intestinal resection. *Am J Clin Nutr*, 54(3), 548-552.
- Lennox, A., Sommerville, J., Ong, K., Henderson, H., & Allen, R. (2013). Diet and nutrition survey of infants and young children, 2011. *A survey carried out on behalf of the Department of Health and Food Standards Agency*.
- Levin, A., Bakris, G., Molitch, M., Smulders, M., Tian, J., Williams, L., & Andress, D. (2007). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*, 71(1), 31-38.
- Levin, A., Bakris, G. L., Molitch, M., Smulders, M., Tian, J., Williams, L. A., & Andress, D. L. (2007). Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*, 71(1), 31-38. doi:10.1038/sj.ki.5002009
- Levin, K. A. (2007). Study design VII. Randomised controlled trials. *J Evid-Based Dent*, 8(1), 22-23.
- Li, P., Li, C., Zhao, X., Zhang, X., Nicosia, S. V., & Bai, W. (2004). p27(Kip1) stabilization and G(1) arrest by 1,25-dihydroxyvitamin D(3) in ovarian cancer cells mediated through down-regulation of cyclin E/cyclin-dependent kinase 2 and Skp1-Cullin-F-box protein/Skp2 ubiquitin ligase. *J Biol Chem*, 279(24), 25260-25267. doi:10.1074/jbc.M311052200
- Li, Y. C. (2003). Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem*, 88(2), 327-331. doi:10.1002/jcb.10343
- Li, Y. C. (2013). Vitamin D receptor signaling in renal and cardiovascular protection. *Semin Nephrol*, 33(5), 433-447. doi:10.1016/j.semnephrol.2013.07.005
- Li, Y. C., Kong, J., Wei, M., Chen, Z. F., Liu, S. Q., & Cao, L. P. (2002). 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*, 110(2), 229-238. doi:10.1172/jci15219

- Libby, P., & Hansson, G. K. (2015). Inflammation and immunity in diseases of the arterial tree players and layers. *Circ Res*, 116(2), 307-311.
- Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature*, 473(7347), 317-325.
- Lieben, L., & Carmeliet, G. (2013). Vitamin D signaling in osteocytes: effects on bone and mineral homeostasis. *Bone*, 54(2), 237-243.
- Lieske, J., & Deganello, S. (1999). Nucleation, adhesion, and internalization of calcium-containing urinary crystals by renal cells. *J Am Soc Nephrol: JASN*, 10, S422-429.
- Lieske, J. C., Pena de la Vega, L. S., Slezak, J. M., Bergstralh, E. J., Leibson, C. L., Ho, K. L., & Gettman, M. T. (2006). Renal stone epidemiology in Rochester, Minnesota: an update. *Kidney Int*, 69(4), 760-764. doi:10.1038/sj.ki.5000150
- Lin, C.-T., Chang, K.-C., Lin, C.-L., Chiang, C.-C., Lu, S.-W., Chang, S.-S., . . . Lee, Y.-T. (2010). An intelligent telecardiology system using a wearable and wireless ECG to detect atrial fibrillation. *IEEE Transactions on Information Technology in Biomedicine*, 14(3), 726-733.
- Lindqvist, A., Sharvill, J., Sharvill, D. E., & Andersson, S. (2007). Loss-of-function mutation in carotenoid 15,15'-monooxygenase identified in a patient with hypercarotenemia and hypovitaminosis A. *J Nutr*, 137(11), 2346-2350.
- Lips, P. (2007). Relative value of 25 (OH) D and 1, 25 (OH) 2D measurements. *J Bone Miner Res*, 22(11), 1668-1671.
- Liu, E., Meigs, J. B., Pittas, A. G., McKeown, N. M., Economos, C. D., Booth, S. L., & Jacques, P. F. (2009). Plasma 25-hydroxyvitamin d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutr*, 139(2), 329-334. doi:10.3945/jn.108.093831
- Liu, M., Lee, M. H., Cohen, M., Bommakanti, M., & Freedman, L. P. (1996). Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. *Genes Dev*, 10(2), 142-153.
- Liu, S., Tang, W., Zhou, J., Stubbs, J. R., Luo, Q., Pi, M., & Quarles, L. D. (2006). Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol*, 17(5), 1305-1315. doi:10.1681/ASN.2005111185

- Liu, Z.-M., Woo, J., Wu, S.-H., & Ho, S. C. (2013). The role of vitamin D in blood pressure, endothelial and renal function in postmenopausal women. *Nutrients*, 5(7), 2590-2610.
- Lokaj, P., Parenica, J., Goldbergova, M. P., Helanová, K., Miklik, R., Kubena, P., . . . Vasku, A. (2011). Pulse pressure in clinical practice. *Eur J Cardiovasc Med*, 2(1), 66-68.
- Lombardi, C. P., Raffaelli, M., Traini, E., Di Stasio, E., Carrozza, C., De Crea, C., . . . Bellantone, R. (2008). Intraoperative PTH monitoring during parathyroidectomy: the need for stricter criteria to detect multiglandular disease. *Langenbecks Arch Surg*, 393(5), 639-645. doi:10.1007/s00423-008-0384-5
- London, G. M., & Guerin, A. P. (1999). Influence of arterial pulse and reflected waves on blood pressure and cardiac function. *Am Heart J*, 138(3 Pt 2), 220-224.
- London, G. M., Guerin, A. P., Verbeke, F. H., Pannier, B., Boutouyrie, P., Marchais, S. J., & Metivier, F. (2007). Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol*, 18(2), 613-620. doi:10.1681/asn.2006060573
- Longenecker, C. T., Hileman, C. O., Carman, T. L., Ross, A. C., Seydafkan, S., Brown, T. T., . . . McComsey, G. A. (2012). Vitamin D supplementation and endothelial function in vitamin D deficient HIV-infected patients: a randomized placebo-controlled trial. *Antivir Ther*, 17(4), 613-621. doi:10.3851/IMP1983
- Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T., & Murray, C. J. (2006). Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *The Lancet*, 367(9524), 1747-1757.
- Lorente-Cebrian, S., Eriksson, A., Dunlop, T., Mejhert, N., Dahlman, I., Astrom, G., . . . Ryden, M. (2012). Differential effects of 1alpha,25-dihydroxycholecalciferol on MCP-1 and adiponectin production in human white adipocytes. *Eur J Nutr*, 51(3), 335-342. doi:10.1007/s00394-011-0218-z
- Lu, S., Guo, S., Hu, F., Guo, Y., Yan, L., Ma, W., . . . Wang, Z. (2016). The Associations Between the Polymorphisms of Vitamin D Receptor and

- Coronary Artery Disease: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)*, 95(21), e3467. doi:10.1097/md.0000000000003467
- Lu, Z., Chen, T. C., Zhang, A., Persons, K. S., Kohn, N., Berkowitz, R., . . . Holick, M. F. (2007). An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? *J Steroid Biochem Mol Biol*, 103(3-5), 642-644. doi:10.1016/j.jsbmb.2006.12.010
- Lynch, T., & Price, A. (2007). The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician*, 76(3), 391-396.
- Maalmi, H., Ordonez-Mena, J. M., Schottker, B., & Brenner, H. (2014). Serum 25-hydroxyvitamin D levels and survival in colorectal and breast cancer patients: systematic review and meta-analysis of prospective cohort studies. *Eur J Cancer*, 50(8), 1510-1521. doi:10.1016/j.ejca.2014.02.006
- MacLaren, R., & Stringer, K. A. (2007). Emerging role of anticoagulants and fibrinolytics in the treatment of acute respiratory distress syndrome. *Pharmacotherapy*, 27(6), 860.
- MacLaughlin, J., & Holick, M. F. (1985). Aging decreases the capacity of human skin to produce vitamin D3. *J Clin Invest*, 76(4), 1536-1538. doi:10.1172/jci112134
- Madar, A. A., Knutsen, K. V., Stene, L. C., Brekke, M., Meyer, H. E., & Lagerlov, P. (2014). Effect of vitamin D3 supplementation on glycated hemoglobin (HbA1c), fructosamine, serum lipids, and body mass index: a randomized, double-blinded, placebo-controlled trial among healthy immigrants living in Norway. *BMJ Open Diabetes Res Care*, 2(1), e000026. doi:10.1136/bmjdr-2014-000026
- Madsen, M. S., Siersbaek, R., Boergesen, M., Nielsen, R., & Mandrup, S. (2014). Peroxisome proliferator-activated receptor gamma and C/EBPalpha synergistically activate key metabolic adipocyte genes by assisted loading. *Mol Cell Biol*, 34(6), 939-954. doi:10.1128/mcb.01344-13
- Maestro, B., Campion, J., Davila, N., & Calle, C. (2000). Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. *Endocr J*, 47(4), 383-391.

- Maislos, M., & Shany, S. (1987). Bile salt deficiency and the absorption of vitamin D metabolites. In vivo study in the rat. *Isr J Med Sci*, 23(11), 1114-1117.
- Maislos, M., Silver, J., & Fainaru, M. (1981). Intestinal absorption of vitamin D sterols: differential absorption into lymph and portal blood in the rat. *Gastroenterology*, 80(6), 1528-1534.
- Maki, K. C., Rubin, M. R., Wong, L. G., McManus, J. F., Jensen, C. D., & Lawless, A. (2011). Effects of vitamin D supplementation on 25-hydroxyvitamin D, high-density lipoprotein cholesterol, and other cardiovascular disease risk markers in subjects with elevated waist circumference. *Int J Food Sci Nutr*, 62(4), 318-327.
- Mallat, Z., Besnard, S., Duriez, M., Deleuze, V., Emmanuel, F., Bureau, M. F., . . . Tedgui, A. (1999). Protective role of interleukin-10 in atherosclerosis. *Circ Res*, 85(8), e17-24.
- Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Böhm, M., . . . Dominiczak, A. (2013). 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Blood pressure*, 22(4), 193-278.
- Mantell, D., Owens, P., Bundred, N., Mawer, E., & Canfield, A. (2000). 1 α , 25-dihydroxyvitamin D₃ inhibits angiogenesis in vitro and in vivo. *Circ Res*, 87(3), 214-220.
- Margolis, R. N., & Christakos, S. (2010). The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation. An update. *Ann N Y Acad Sci*, 1192, 208-214. doi:10.1111/j.1749-6632.2009.05227.x
- Marti, C. N., Gheorghiade, M., Kalogeropoulos, A. P., Georgiopoulou, V. V., Quyyumi, A. A., & Butler, J. (2012). Endothelial dysfunction, arterial stiffness, and heart failure. *J Am Coll Cardiol*, 60(16), 1455-1469.
- Martin-Ventura, J. L., Ortego, M., Esbrit, P., Hernandez-Presa, M. A., Ortega, L., & Egido, J. (2003). Possible role of parathyroid hormone-related protein as a proinflammatory cytokine in atherosclerosis. *Stroke*, 34(7), 1783-1789. doi:10.1161/01.STR.0000078371.00577.76
- Martin, T., & Campbell, R. K. (2011). Vitamin D and diabetes. *Diabetes Spectrum*, 24(2), 113-118.

- Martins, D., Meng, Y. X., Tareen, N., Artaza, J., Lee, J. E., Farodolu, C., . . . Norris, K. (2014). The Effect of Short Term Vitamin D Supplementation on the Inflammatory and Oxidative Mediators of Arterial Stiffness. *Health (Irvine Calif)*, 6(12), 1503-1511. doi:10.4236/health.2014.612185
- Masterson, J. H., Woo, J. R., Chang, D. C., Chi, T., L'Esperance, J. O., Stoller, M. L., & Sur, R. L. (2015). Dyslipidemia is associated with an increased risk of nephrolithiasis. *Urolithiasis*, 43(1), 49-53. doi:10.1007/s00240-014-0719-3
- Masuyama, R., Nakaya, Y., Katsumata, S., Kajita, Y., Uehara, M., Tanaka, S., . . . Suzuki, K. (2003). Dietary calcium and phosphorus ratio regulates bone mineralization and turnover in vitamin D receptor knockout mice by affecting intestinal calcium and phosphorus absorption. *J Bone Miner Res*, 18(7), 1217-1226. doi:10.1359/jbmr.2003.18.7.1217
- Matkovits, T., & Christakos, S. (1995). Variable in vivo regulation of rat vitamin D-dependent genes (osteopontin, Ca,Mg-adenosine triphosphatase, and 25-hydroxyvitamin D3 24-hydroxylase): implications for differing mechanisms of regulation and involvement of multiple factors. *Endocrinology*, 136(9), 3971-3982. doi:10.1210/endo.136.9.7649106
- Matsuda, M., & Shimomura, I. (2013). Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract*, 7(5), e330-341.
- Matsuoka, L. Y., Wortsman, J., Hanifan, N., & Holick, M. F. (1988). Chronic sunscreen use decreases circulating concentrations of 25-hydroxyvitamin D. A preliminary study. *Arch Dermatol*, 124(12), 1802-1804.
- Mattoo, A., & Goldfarb, D. S. (2008). Cystinuria. *Semin Nephrol*, 28(2), 181-191. doi:10.1016/j.semnephrol.2008.01.011
- Maury, E., & Brichard, S. M. (2010). Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol*, 314(1), 1-16. doi:10.1016/j.mce.2009.07.031
- Mayer, O., Filipovský, J., Seidlerová, J., Vaněk, J., Dolejšová, M., Vrzalová, J., & Cífková, R. (2012). The association between low 25-hydroxyvitamin D and increased aortic stiffness. *J Hum Hypertens*, 26(11), 650-655.
- McCord, J. M., & Edeas, M. A. (2005). SOD, oxidative stress and human pathologies: a brief history and a future vision. *Biomed Pharmacother*, 59(4), 139-142. doi:10.1016/j.biopha.2005.03.005

- McCullough, M. L., Bostick, R. M., & Mayo, T. L. (2009). Vitamin D gene pathway polymorphisms and risk of colorectal, breast, and prostate cancer. *Ann Rev Nutr*, 29, 111-132.
- McDuffie, J. R., Calis, K. A., Booth, S. L., Uwaifo, G. I., & Yanovski, J. A. (2002). Effects of orlistat on fat-soluble vitamins in obese adolescents. *Pharmacotherapy*, 22(7), 814-822.
- McGorisk, G. M., & Treasure, C. B. (1996). Endothelial dysfunction in coronary heart disease. *Curr Opin Cardiol*, 11(4), 341-350.
- McGreevy, C., Barry, M., Davenport, C., Byrne, B., Donaghy, C., Collier, G., . . . Williams, D. (2015). The effect of vitamin D supplementation on arterial stiffness in an elderly community-based population. *J Am Soc Hypertens*, 9(3), 176-183.
- Melamed, M. L., Michos, E. D., Post, W., & Astor, B. (2008). 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med*, 168(15), 1629-1637. doi:10.1001/archinte.168.15.1629
- Menezes, A. R., Lamb, M. C., Lavie, C. J., & DiNicolantonio, J. J. (2014). Vitamin D and atherosclerosis. *Curr Opin Cardiol*, 29(6), 571-577.
- Mezza, T., Muscogiuri, G., Sorice, G. P., Prioletta, A., Salomone, E., Pontecorvi, A., & Giaccari, A. (2012). Vitamin D deficiency: a new risk factor for type 2 diabetes? *Ann Nutr Metab*, 61(4), 337-348. doi:10.1159/000342771
- Miettinen, M. E., Kinnunen, L., Leiviskä, J., Keinänen-Kiukaanniemi, S., Korpi-Hyövälti, E., Niskanen, L., . . . Vanhala, M. (2014). Association of serum 25-hydroxyvitamin D with lifestyle factors and metabolic and cardiovascular disease markers: population-based cross-sectional study (FIN-D2D). *PLoS One*, 9(7), e100235.
- Milne, G. L., Yin, H., Brooks, J. D., Sanchez, S., Roberts, L. J., & Morrow, J. D. (2007). Quantification of F2-Isoprostanes in Biological Fluids and Tissues as a Measure of Oxidant Stress. *Meth Enzymol*, 433, 113-126.
- Mitri, J., Dawson-Hughes, B., Hu, F. B., & Pittas, A. G. (2011). Effects of vitamin D and calcium supplementation on pancreatic beta cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr*, 94(2), 486-494. doi:10.3945/ajcn.111.011684

- Moan, J., Porojnicu, A. C., Dahlback, A., & Setlow, R. B. (2008). Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. *Proc Natl Acad Sci U S A*, 105(2), 668-673. doi:10.1073/pnas.0710615105
- Moe, O. W. (2006). Kidney stones: pathophysiology and medical management. *The Lancet*, 367(9507), 333-344.
- Moe, O. W., Abate, N., & Sakhaee, K. (2002). Pathophysiology of uric acid nephrolithiasis. *Endocrinol Metab Clin North Am*, 31(4), 895-914.
- Moe, S. M. (2008). Disorders involving calcium, phosphorus, and magnesium. *Primary Care: Clinics in Office Practice*, 35(2), 215-237.
- Moe, S. M., & Chen, N. X. (2005). Inflammation and vascular calcification. *Blood Purif*, 23(1), 64-71.
- Moe, S. M., & Chen, N. X. (2008). Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol*, 19(2), 213-216.
- Monraats, P. S., Fang, Y., Pons, D., Pires, N. M., Pols, H. A., Zwinderman, A. H., . . . Jukema, J. W. (2010). Vitamin D receptor: a new risk marker for clinical restenosis after percutaneous coronary intervention. *Expert Opin Ther Targets*, 14(3), 243-251. doi:10.1517/14728220903520929
- Montagnani, M., Lore, F., Di Cairano, G., Gonnelli, S., Ciuoli, C., Montagnani, A., & Gennari, C. (1994). Effects of pravastatin treatment on vitamin D metabolites. *Clin Ther*, 16(5), 824-829.
- Moradi, H., Vaziri, N. D., Kashyap, M. L., Said, H. M., & Kalantar-Zadeh, K. (2013). Role of HDL dysfunction in end-stage renal disease: a double-edged sword. *J Ren Nutr*, 23(3), 203-206. doi:10.1053/j.jrn.2013.01.022
- Morris, S. M., Tallquist, M. D., Rock, C. O., & Cooper, J. A. (2002). Dual roles for the Dab2 adaptor protein in embryonic development and kidney transport. *EMBO J*, 21(7), 1555-1564. doi:10.1093/emboj/21.7.1555
- Motiwalla, S. R., & Wang, T. J. (2012). Vitamin D and cardiovascular risk. *Curr Hypertens Rep*, 14(3), 209-218. doi:10.1007/s11906-012-0262-y
- Mozaffari-Khosravi, H., Loloei, S., Mirjalili, M.-R., & Barzegar, K. (2015). The effect of vitamin D supplementation on blood pressure in patients with elevated blood pressure and vitamin D deficiency: a randomized, double-blind, placebo-controlled trial. *Blood pressure monitoring*, 20(2), 83-91.

- Mozos, I., & Marginean, O. (2015). Links between Vitamin D Deficiency and Cardiovascular Diseases. *Biomed Res Int*, 2015, 109275. doi:10.1155/2015/109275
- Muller, K., Haahr, P. M., Diamant, M., Rieneck, K., Kharazmi, A., & Bendtzen, K. (1992). 1,25-Dihydroxyvitamin D3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. *Cytokine*, 4(6), 506-512.
- Mulligan, G. B., & Licata, A. (2010). Taking vitamin D with the largest meal improves absorption and results in higher serum levels of 25-hydroxyvitamin D. *J Bone Miner Res*, 25(4), 928-930. doi:10.1002/jbmr.67
- Muniyappa, R., & Yavuz, S. (2013). Metabolic actions of angiotensin II and insulin: a microvascular endothelial balancing act. *Mol Cell Endocrinol*, 378(1), 59-69.
- Munzel, T., Sinning, C., Post, F., Warnholtz, A., & Schulz, E. (2008). Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Ann Med*, 40(3), 180-196. doi:10.1080/07853890701854702
- Muscogiuri, G., Sorice, G. P., Ajjan, R., Mezza, T., Pilz, S., Priolella, A., . . . Giaccari, A. (2012). Can vitamin D deficiency cause diabetes and cardiovascular diseases? Present evidence and future perspectives. *Nutr Metab Cardiovasc Dis*, 22(2), 81-87. doi:10.1016/j.numecd.2011.11.001
- Nadar, S., Blann, A. D., & Lip, G. Y. (2004). Endothelial dysfunction: methods of assessment and application to hypertension. *Curr Pharm Des*, 10(29), 3591-3605.
- Naghavi, M., Wang, H., Lozano, R., Davis, A., Liang, X., Zhou, M., . . . Abd-Allah, F. (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, 385(9963), 117-171.
- Nagpal, J., Pande, J. N., & Bhartia, A. (2009). A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. *Diabet Med*, 26(1), 19-27. doi:10.1111/j.1464-5491.2008.02636.x
- Nagpal, S., Na, S., & Rathnachalam, R. (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev*, 26(5), 662-687. doi:10.1210/er.2004-0002

- Najeeb, Q., Masood, I., Bhaskar, N., Kaur, H., Singh, J., Pandey, R., . . . Mahajan, R. (2013). Effect of BMI and urinary pH on urolithiasis and its composition. *Saudi J Kidney Dis Transpl*, 24(1), 60-66.
- Nakamura, S., Ishibashi-Ueda, H., Niizuma, S., Yoshihara, F., Horio, T., & Kawano, Y. (2009). Coronary calcification in patients with chronic kidney disease and coronary artery disease. *Clin J Am Soc Nephrol*, CJN. 04320709.
- Nakano, T., Ninomiya, T., Sumiyoshi, S., Fujii, H., Doi, Y., Hirakata, H., . . . Sueishi, K. (2010). Association of kidney function with coronary atherosclerosis and calcification in autopsy samples from Japanese elders: the Hisayama study. *Am J Kidney Dis*, 55(1), 21-30. doi:10.1053/j.ajkd.2009.06.034
- Nanayakkara, P. W., Teerlink, T., Stehouwer, C. D., Allajar, D., Spijkerman, A., Schalkwijk, C., . . . van Guldener, C. (2005). Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. *Kidney Int*, 68(5), 2230-2236. doi:10.1111/j.1523-1755.2005.00680.x
- National Institute for Health and Clinical Excellence. (2007). PH11 maternal and child nutrition: supporting evidence review 7. The effectiveness and cost-effectiveness of interventions to promote an optimal intake of Vitamin D to improve the nutrition of pre-conceptual, pregnant and post-partum women and children, in low income households. Retrieved from <http://www.nice.org.uk/nicemedia/live/11943/43901/43901.pdf>
- National Osteoporosis Society. (2013). *Vitamin D and bone health: a practical clinical guideline for patient management*. Retrieved from <https://nos.org.uk/media/2073/vitamin-d-and-bone-health-adults.pdf>.
- Nesby-O'Dell, S., Scanlon, K. S., Cogswell, M. E., Gillespie, C., Hollis, B. W., Looker, A. C., . . . Bowman, B. A. (2002). Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr*, 76(1), 187-192.
- Neyestani, T. R., Djazayeri, A., Shab-Bidar, S., Eshraghian, M. R., Kalayi, A., Shariatzadeh, N., . . . Asadzadeh, S. (2013). Vitamin D Receptor Fok-I polymorphism modulates diabetic host response to vitamin D intake: need for

- a nutrigenetic approach. *Diabetes Care*, 36(3), 550-556. doi:10.2337/dc12-0919
- Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., . . . Abera, S. F. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, 384(9945), 766-781.
- Ngo, D. T., Sverdlov, A. L., McNeil, J. J., & Horowitz, J. D. (2010). Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? *Am J Med*, 123(4), 335-341. doi:10.1016/j.amjmed.2009.09.024
- Nigwekar, S. U., Bhan, I., & Thadhani, R. (2012). Ergocalciferol and cholecalciferol in CKD. *Am J Kidney Dis*, 60(1), 139-156. doi:10.1053/j.ajkd.2011.12.035
- Nigwekar, S. U., Tamez, H., & Thadhani, R. I. (2014). Vitamin D and chronic kidney disease-mineral bone disease (CKD-MBD). *Bonekey Rep*, 3, 498. doi:10.1038/bonekey.2013.232
- Nikooyeh, B., & Neyestani, T. R. (2016). Oxidative stress, type 2 diabetes and vitamin D: past, present and future. *Diabetes Metab Res Rev*, 32(3), 260-267. doi:10.1002/dmrr.2718
- Nishio, S., Hatanaka, M., Takeda, H., Aoki, K., Iseda, T., Iwata, H., & Yokoyama, M. (2000). Calcium phosphate crystal-associated proteins: alpha2-HS-glycoprotein, prothrombin F1, and osteopontin. *Mol Urol*, 4(4), 383-390.
- Nishizawa, Y., Shoji, T., Kawagishi, T., & Morii, H. (1997). Atherosclerosis in uremia: possible roles of hyperparathyroidism and intermediate density lipoprotein accumulation. *Kidney Int Suppl*, 62, S90-92.
- Nnoaham, K. E., & Clarke, A. (2008). Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol*, 37(1), 113-119. doi:10.1093/ije/dym247
- Norman, A. W. (2008). From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr*, 88(2), 491S-499S.
- Norman, P. E., & Powell, J. T. (2005). Vitamin D, shedding light on the development of disease in peripheral arteries. *Arterioscler Thromb Vasc Biol*, 25(1), 39-46. doi:10.1161/01.ATV.0000148450.56697.4a

- Norval, M., Bjorn, L. O., & de Gruijl, F. R. (2010). Is the action spectrum for the UV-induced production of previtamin D3 in human skin correct? *Photochem Photobiol Sci*, 9(1), 11-17. doi:10.1039/b9pp00012g
- Nwudu, V. C. (2015). Association between obesity and vitamin D deficiency. *Obes Rev*, 16(9), 817. doi:10.1111/obr.12291
- Nykjaer, A., Dragun, D., Walther, D., Vorum, H., Jacobsen, C., Herz, J., . . . Willnow, T. E. (1999). An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell*, 96(4), 507-515.
- Nykjaer, A., Fyfe, J. C., Kozyraki, R., Leheste, J. R., Jacobsen, C., Nielsen, M. S., . . . Christensen, E. I. (2001). Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D(3). *Proc Natl Acad Sci U S A*, 98(24), 13895-13900. doi:10.1073/pnas.241516998
- O'Brien, E. R., Garvin, M. R., Stewart, D. K., Hinohara, T., Simpson, J. B., Schwartz, S. M., & Giachelli, C. M. (1994). Osteopontin is synthesized by macrophage, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques. *Arterioscler Thromb*, 14(10), 1648-1656.
- O'rourke, M. F., & Hashimoto, J. (2007). Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol*, 50(1), 1-13.
- Ochs-Balcom, H. M., Chennamaneni, R., Millen, A. E., Shields, P. G., Marian, C., Trevisan, M., & Freudenheim, J. L. (2011). Vitamin D receptor gene polymorphisms are associated with adiposity phenotypes. *Am J Clin Nutr*, 93(1), 5-10.
- Odabaei, G., Kaysen, G. A., & Ananthakrishnan, S. (2014). Chronic Kidney Disease *Metabolism of Human Diseases* (pp. 345-350): Springer.
- Oh, J., Weng, S., Felton, S. K., Bhandare, S., Riek, A., Butler, B., . . . Bernal-Mizrachi, C. (2009). 1,25(OH)₂ vitamin d inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. *Circulation*, 120(8), 687-698. doi:10.1161/CIRCULATIONAHA.109.856070
- Olszanecka-Glinianowicz, M., Zahorska-Markiewicz, B., Janowska, J., & Zurakowski, A. (2004). Serum concentrations of nitric oxide, tumor necrosis factor (TNF)- α and TNF soluble receptors in women with overweight and obesity. *Metabolism*, 53(10), 1268-1273.

- Orr, A. W., Yurdagul Jr, A., & Patel, B. M. (2014). *Pathogenesis of Atherosclerosis: From Cell Biology to Therapeutics*. Paper presented at the Colloquium Series on Integrated Systems Physiology: From Molecule to Function to Disease.
- Ortega, R. M., Perez-Rodrigo, C., & Lopez-Sobaler, A. M. (2015). Dietary assessment methods: dietary records. *Nutr Hosp*, 31 Suppl 3, 38-45. doi:10.3305/nh.2015.31.sup3.8749
- Otani, H. (2011). Oxidative stress as pathogenesis of cardiovascular risk associated with metabolic syndrome. *Antioxid Redox Signal*, 15(7), 1911-1926. doi:10.1089/ars.2010.3739
- Palombo, C., & Kozakova, M. (2016). Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. *Vascul Pharmacol*, 77, 1-7. doi:10.1016/j.vph.2015.11.083
- Parker, J., Hashmi, O., Dutton, D., Mavrodaris, A., Stranges, S., Kandala, N.-B., . . . Franco, O. H. (2010). Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. *Maturitas*, 65(3), 225-236.
- Parks, J. H., Worcester, E. M., Coe, F. L., Evan, A. P., & Lingeman, J. E. (2004). Clinical implications of abundant calcium phosphate in routinely analyzed kidney stones. *Kidney Int*, 66(2), 777-785. doi:10.1111/j.1523-1755.2004.00803.x
- Patel, S. R., Ke, H. Q., Vanholder, R., Koenig, R. J., & Hsu, C. H. (1995). Inhibition of calcitriol receptor binding to vitamin D response elements by uremic toxins. *J Clin Invest*, 96(1), 50-59. doi:10.1172/jci118061
- Pathak, K., Soares, M. J., Calton, E. K., Zhao, Y., & Hallett, J. (2014). Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev*, 15(6), 528-537. doi:10.1111/obr.12162
- Peng, J. B., Brown, E. M., & Hediger, M. A. (2003). Apical entry channels in calcium-transporting epithelia. *News Physiol Sci*, 18, 158-163.
- Pereira-Santos, M., Costa, P. R., Assis, A. M., Santos, C. A., & Santos, D. B. (2015). Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obes Rev*, 16(4), 341-349. doi:10.1111/obr.12239
- Perrotta, I., & Aquila, S. (2015). The role of oxidative stress and autophagy in atherosclerosis. *Oxid Med Cell longev*, 2015.

- Perwad, F., & Portale, A. A. (2011). Vitamin D metabolism in the kidney: regulation by phosphorus and fibroblast growth factor 23. *Mol Cell Endocrinol*, 347(1-2), 17-24. doi:10.1016/j.mce.2011.08.030
- Peterson, C. A., & Heffernan, M. E. (2008). Serum tumor necrosis factor- α concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *J Inflamm (Lond)*, 5, 10. doi:10.1186/1476-9255-5-10
- Petkovich, M., & Jones, G. (2011). CYP24A1 and kidney disease. *Curr Opin Nephrol Hypertens*, 20(4), 337-344. doi:10.1097/MNH.0b013e3283477a7b
- Pfeifer, M., Begerow, B., & Minne, H. W. (2002). Vitamin D and muscle function. *Osteoporos Int*, 13(3), 187-194. doi:10.1007/s001980200012
- Pike, J. W., & Meyer, M. B. (2010). The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). *Endocrinol Metab Clin North Am*, 39(2), 255-269, table of contents. doi:10.1016/j.ecl.2010.02.007
- Pilz, S., Gaksch, M., Kienreich, K., Grubler, M., Verheyen, N., Fahrleitner-Pammer, A., . . . Tomaschitz, A. (2015). Effects of vitamin D on blood pressure and cardiovascular risk factors: a randomized controlled trial. *Hypertension*, 65(6), 1195-1201. doi:10.1161/hypertensionaha.115.05319
- Pilz, S., Tomaschitz, A., März, W., Drechsler, C., Ritz, E., Zittermann, A., . . . Grant, W. B. (2011). Vitamin D, cardiovascular disease and mortality. *Clin Endocrinol (Oxf)*, 75(5), 575-584.
- Pilz, S., Tomaschitz, A., Ritz, E., & Pieber, T. R. (2009). Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol*, 6(10), 621-630.
- Pini, R., Cavallini, M. C., Palmieri, V., Marchionni, N., Di Bari, M., Devereux, R. B., . . . Roman, M. J. (2008). Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. *J Am Coll Cardiol*, 51(25), 2432-2439.
- Pipili, C., & Oreopoulos, D. G. (2012). Vitamin D status in patients with recurrent kidney stones. *Nephron Clin Pract*, 122(3-4), 134-138. doi:10.1159/000351377
- Pirro, M., Manfredelli, M. R., Helou, R. S., Scarponi, A. M., Schillaci, G., Bagaglia, F., . . . Mannarino, E. (2012). Association of parathyroid hormone and 25-OH-vitamin D levels with arterial stiffness in postmenopausal women with vitamin D insufficiency. *J Atheroscler Thromb*, 19(10), 924-931.

- Pittas, A. G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., Patel, K., . . . Balk, E. M. (2010). Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med*, 152(5), 307-314. doi:10.7326/0003-4819-152-5-201003020-00009
- Pittas, A. G., Joseph, N. A., & Greenberg, A. S. (2004). Adipocytokines and insulin resistance. *J Clin Endocrinol Metab*, 89(2), 447-452. doi:10.1210/jc.2003-031005
- Pittas, A. G., Lau, J., Hu, F. B., & Dawson-Hughes, B. (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*, 92(6), 2017-2029. doi:10.1210/jc.2007-0298
- Pludowski, P., Karczmarewicz, E., Bayer, M., Carter, G., Chlebna-Sokol, D., Czech-Kowalska, J., . . . Zmijewski, M. A. (2013). Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe - recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. *Endokrynol Pol*, 64(4), 319-327.
- Ponticos, M., & Smith, B. D. (2014). Extracellular matrix synthesis in vascular disease: hypertension, and atherosclerosis. *J Biomed Res*, 28(1), 25-39.
- Pontiroli, A. E., Frige, F., Paganelli, M., & Folli, F. (2009). In morbid obesity, metabolic abnormalities and adhesion molecules correlate with visceral fat, not with subcutaneous fat: effect of weight loss through surgery. *Obes Surg*, 19(6), 745-750. doi:10.1007/s11695-008-9626-4
- Potthoff, S. A., Janus, A., Hoch, H., Frahnert, M., Tossios, P., Reber, D., . . . Vonend, O. (2011). PTH-receptors regulate norepinephrine release in human heart and kidney. *Regul Pept*, 171(1-3), 35-42. doi:10.1016/j.regpep.2011.06.002
- Powe, C. E., Evans, M. K., Wenger, J., Zonderman, A. B., Berg, A. H., Nalls, M., . . . Karumanchi, S. A. (2013). Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med*, 369(21), 1991-2000.
- Pradhan, A. (2007). Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. *Nutr Rev*, 65(12 Pt 2), S152-156.
- Prentice, A. (2002). What are the dietary requirements for calcium and vitamin D? *Calcif Tissue Int*, 70(2), 83-88. doi:10.1007/s00223-001-0035-0

- Public Health England. (2015). NHS Diabetes Prevention Programme (NHS DPP) Non-diabetic hyperglycaemia. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/456149/Non_diabetic_hyperglycaemia.pdf
- Public Health England. (2016). National Diet and Nutrition Survey. Results from Year 5 and 6 combined. Retrieved from <https://www.gov.uk/government/statistics/ndns-results-from-years-5-and-6-combined>
- Quarles, L. D. (2012). Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. *Exp Cell Res*, 318(9), 1040-1048. doi:10.1016/j.yexcr.2012.02.027
- Raghuwanshi, A., Joshi, S. S., & Christakos, S. (2008). Vitamin D and multiple sclerosis. *J Cell Biochem*, 105(2), 338-343. doi:10.1002/jcb.21858
- Rashid, S., & Genest, J. (2007). Effect of Obesity on High-density Lipoprotein Metabolism. *Obesity (Silver Spring)*, 15(12), 2875-2888.
- Ratkalkar, V. N., & Kleinman, J. G. (2011). Mechanisms of Stone Formation. *Clin Rev Bone Miner Metab*, 9(3-4), 187-197. doi:10.1007/s12018-011-9104-8
- Reboul, E. (2015). Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food Funct*, 6(2), 356-362. doi:10.1039/c4fo00579a
- Reboul, E., Berton, A., Moussa, M., Kreuzer, C., Crenon, I., & Borel, P. (2006). Pancreatic lipase and pancreatic lipase-related protein 2, but not pancreatic lipase-related protein 1, hydrolyze retinyl palmitate in physiological conditions. *Biochim Biophys Acta*, 1761(1), 4-10. doi:10.1016/j.bbalip.2005.12.013
- Reboul, E., Goncalves, A., Comera, C., Bott, R., Nowicki, M., Landrier, J. F., . . . Borel, P. (2011). Vitamin D intestinal absorption is not a simple passive diffusion: evidences for involvement of cholesterol transporters. *Mol Nutr Food Res*, 55(5), 691-702. doi:10.1002/mnfr.201000553
- Reid, D. G., Jackson, G. J., Duer, M. J., & Rodgers, A. L. (2011). Apatite in kidney stones is a molecular composite with glycosaminoglycans and proteins: evidence from nuclear magnetic resonance spectroscopy, and relevance to Randall's plaque, pathogenesis and prophylaxis. *J Urol*, 185(2), 725-730. doi:10.1016/j.juro.2010.09.075

- Replogle, R. A., Li, Q., Wang, L., Zhang, M., & Fleet, J. C. (2014). Gene-by-diet interactions influence calcium absorption and bone density in mice. *J Bone Miner Res*, 29(3), 657-665. doi:10.1002/jbmr.2065
- Reshetnik, A., Gohlisch, C., Zidek, W., Tölle, M., & van der Giet, M. (2016). Validation of the Tel-O-GRAPE, a new oscillometric blood pressure-measuring device, according to the British Hypertension Society protocol. *Blood pressure monitoring*.
- Reymond, N., d'Água, B. B., & Ridley, A. J. (2013). Crossing the endothelial barrier during metastasis. *Nature Reviews Cancer*, 13(12), 858-870.
- Riachy, R., Vandewalle, B., Kerr Conte, J., Moerman, E., Sacchetti, P., Lukowiak, B., . . . Pattou, F. (2002). 1,25-dihydroxyvitamin D3 protects RINm5F and human islet cells against cytokine-induced apoptosis: implication of the antiapoptotic protein A20. *Endocrinology*, 143(12), 4809-4819. doi:10.1210/en.2002-220449
- Riccardi, D., & Brown, E. M. (2010). Physiology and pathophysiology of the calcium-sensing receptor in the kidney. *Am J Physiol-Ren Physiol*, 298(3), F485-F499.
- Riek, A. E., Oh, J., & Bernal-Mizrachi, C. (2013). 1, 25 (OH) 2 vitamin D suppresses macrophage migration and reverses atherogenic cholesterol metabolism in type 2 diabetic patients. *J Steroid Biochem Mol Biol*, 136, 309-312.
- Ritz, E., & Wanner, C. (2008). Lipid abnormalities and cardiovascular risk in renal disease. *J Am Soc Nephrol*, 19(6), 1065-1070.
- Rivera-Leon, E. A., Palmeros-Sanchez, B., Llama-Covarrubias, I. M., Fernandez, S., Armendariz-Borunda, J., Gonzalez-Hita, M., . . . Sanchez-Enriquez, S. (2015). Vitamin-D receptor gene polymorphisms (TaqI and ApaI) and circulating osteocalcin in type 2 diabetic patients and healthy subjects. *Endokrynol Pol*, 66(4), 329-333. doi:10.5603/ep.2015.0042
- Robberecht, E., Vandewalle, S., Wehlou, C., Kaufman, J. M., & De Schepper, J. (2011). Sunlight is an important determinant of vitamin D serum concentrations in cystic fibrosis. *Eur J Clin Nutr*, 65(5), 574-579. doi:10.1038/ejcn.2010.280
- Roberts, W. (2013). *Assessment of Coronary Artery Disease by Computed Tomography*. Queen Mary University of London.

- Robinson-Bostom, L., & DiGiovanna, J. J. (2000). Cutaneous manifestations of end-stage renal disease. *J Am Acad Dermatol*, 43(6), 975-986.
- Rochel, N., Wurtz, J. M., Mitschler, A., Klaholz, B., & Moras, D. (2000). The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol Cell*, 5(1), 173-179.
- Rodriguez, A. J., Scott, D., Srikanth, V., & Ebeling, P. (2016). Effect of vitamin D supplementation on measures of arterial stiffness: a systematic review and meta-analysis of randomized controlled trials. *Clin Endocrinol (Oxf)*, 84(5), 645-657. doi:10.1111/cen.13031
- Rojas, A., Romay, S., González, D., Herrera, B., Delgado, R., & Otero, K. (2000). Regulation of endothelial nitric oxide synthase expression by albumin-derived advanced glycosylation end products. *Circ Res*, 86(3), e50-e54.
- Romacho, T., Elsen, M., Rohrborn, D., & Eckel, J. (2014). Adipose tissue and its role in organ crosstalk. *Acta Physiol (Oxf)*, 210(4), 733-753. doi:10.1111/apha.12246
- Romagnoli, E., Mascia, M. L., Cipriani, C., Fassino, V., Mazzei, F., D'Erasmus, E., . . . Minisola, S. (2008). Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *J Clin Endocrinol Metab*, 93(8), 3015-3020. doi:10.1210/jc.2008-0350
- Romero, V., Akpınar, H., & Assimos, D. G. (2010). Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev Urol*, 12(2-3), e86-96.
- Rosen, C. J., Adams, J. S., Bikle, D. D., Black, D. M., Demay, M. B., Manson, J. E., . . . Kovacs, C. S. (2012). The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev*, 33(3), 456-492.
- Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., . . . Jones, G. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metabol*, 96(1), 53-58.
- Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med*, 340(2), 115-126. doi:10.1056/NEJM199901143400207

- Rule, A. D., Bergstralh, E. J., Melton, L. J., 3rd, Li, X., Weaver, A. L., & Lieske, J. C. (2009). Kidney stones and the risk for chronic kidney disease. *Clin J Am Soc Nephrol*, 4(4), 804-811. doi:10.2215/CJN.05811108
- Rule, A. D., Krambeck, A. E., & Lieske, J. C. (2011). Chronic kidney disease in kidney stone formers. *Clin J Am Soc Nephrol*, 6(8), 2069-2075.
- Rule, A. D., Roger, V. L., Melton, L. J., Bergstralh, E. J., Li, X., Peyser, P. A., . . . Lieske, J. C. (2010). Kidney stones associate with increased risk for myocardial infarction. *J Am Soc Nephrol*, 21(10), 1641-1644.
- Safadi, F. F., Thornton, P., Magiera, H., Hollis, B. W., Gentile, M., Haddad, J. G., . . . Cooke, N. E. (1999). Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein. *J Clin Invest*, 103(2), 239-251. doi:10.1172/JCI5244
- Sakhaee, K. (2009). Recent advances in the pathophysiology of nephrolithiasis. *Kidney Int*, 75(6), 585-595. doi:10.1038/ki.2008.626
- Sakhaee, K., Maalouf, N. M., & Sinnott, B. (2012). Clinical review. Kidney stones 2012: pathogenesis, diagnosis, and management. *J Clin Endocrinol Metab*, 97(6), 1847-1860. doi:10.1210/jc.2011-3492
- Saleh, F. N., Schirmer, H., Sundsfjord, J., & Jorde, R. (2003). Parathyroid hormone and left ventricular hypertrophy. *Eur Heart J*, 24(22), 2054-2060.
- Salehpour, A., Hosseinpanah, F., Shidfar, F., Vafa, M., Razaghi, M., Dehghani, S., . . . Gohari, M. (2012). A 12-week double-blind randomized clinical trial of vitamin D(3) supplementation on body fat mass in healthy overweight and obese women. *Nutr J*, 11, 78. doi:10.1186/1475-2891-11-78
- Salekzamani, S., Mehralizadeh, H., Ghezel, A., Salekzamani, Y., Jafarabadi, M., Babil, A., & Gargari, B. (2016). Effect of high-dose vitamin D supplementation on cardiometabolic risk factors in subjects with metabolic syndrome: a randomized controlled double-blind clinical trial. *J Endocrinol Invest*, 39(11), 1303-1313.
- Sandersius, S., & Rez, P. (2007). Morphology of crystals in calcium oxalate monohydrate kidney stones. *Urol Res*, 35(6), 287-293. doi:10.1007/s00240-007-0115-3
- Sattler, K., & Levkau, B. (2009). Sphingosine-1-phosphate as a mediator of high-density lipoprotein effects in cardiovascular protection. *Cardiovasc Res*, 82(2), 201-211.

- Sayed-Hassan, R., Abazid, N., & Alourfi, Z. (2014). Relationship between 25-hydroxyvitamin D concentrations, serum calcium, and parathyroid hormone in apparently healthy Syrian people. *Arch Osteoporos*, 9, 176. doi:10.1007/s11657-014-0176-1
- Sayers, J., Hynes, A., Rice, S., Hogg, P., & Sayer, J. (2013). Searching for CYP24A1 mutations in cohorts of patients with calcium nephrolithiasis. *OA Nephrology*, 1(1), 6.
- Scagliusi, F. B., Polacow, V. O., Artioli, G. G., Benatti, F. B., & Lancha, A. H. (2003). Selective underreporting of energy intake in women: magnitude, determinants, and effect of training. *J Am Diet Assoc*, 103(10), 1306-1313.
- Schauber, J., Dorschner, R. A., Coda, A. B., Buchau, A. S., Liu, P. T., Kiken, D., . . . Gallo, R. L. (2007). Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest*, 117(3), 803-811. doi:10.1172/jci30142
- Schmid, A., & Walther, B. (2013). Natural vitamin D content in animal products. *Adv Nutr*, 4(4), 453-462. doi:10.3945/an.113.003780
- Schmidt, D. R., Holmstrom, S. R., Fon Tacer, K., Bookout, A. L., Kliwer, S. A., & Mangelsdorf, D. J. (2010). Regulation of bile acid synthesis by fat-soluble vitamins A and D. *J Biol Chem*, 285(19), 14486-14494. doi:10.1074/jbc.M110.116004
- Schroten, N. F., Ruifrok, W. P., Kleijn, L., Dokter, M. M., Silljé, H. H., Heerspink, H. J. L., . . . van Veldhuisen, D. J. (2013). Short-term vitamin D3 supplementation lowers plasma renin activity in patients with stable chronic heart failure: an open-label, blinded end point, randomized prospective trial (VitD-CHF trial). *Am Heart J*, 166(2), 357-364. e352.
- Schulz, K. F., Altman, D. G., Moher, D., & Group, C. (2010). CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials. *J Clin Epidemiol*, 63(8), 834-840. doi:10.1016/j.jclinepi.2010.02.005
- Schurgers, L. J., Dissel, P. E., Spronk, H. M., Soute, B. A., Dhore, C. R., Cleutjens, J. P., & Vermeer, C. (2001). Role of vitamin K and vitamin K-dependent proteins in vascular calcification. *Z Kardiol*, 90 Suppl 3, 57-63.
- Schwalfenberg, G. K., & Genuis, S. J. (2010). Vitamin D supplementation in a nursing home population. *Mol Nutr Food Res*, 54(8), 1072-1076. doi:10.1002/mnfr.200900601

- Scientific Advisory Committee on Nutrition. (2007). *Update on vitamin D. Position statement*: London: The Stationary Office.
- Scientific Advisory Committee on Nutrition. (2015). Vitamin D and Health report. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/447402/Draft_SACN_Vitamin_D_and_Health_Report.pdf
- Scientific Advisory Committee on Nutrition. (2016). Vitamin D and Health Report. Retrieved from <https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report>
- Scragg, R., Jackson, R., Holdaway, I. M., Lim, T., & Beaglehole, R. (1990). Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. *Int J Epidemiol*, 19(3), 559-563.
- Scragg, R., Sowers, M., & Bell, C. (2007). Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens*, 20(7), 713-719.
- Sebekova, K., Sturmer, M., Fazeli, G., Bahner, U., Stab, F., & Heidland, A. (2015). Is vitamin D deficiency related to accumulation of advanced glycation end products, markers of inflammation, and oxidative stress in diabetic subjects? *Biomed Res Int*, 2015, 958097. doi:10.1155/2015/958097
- Segaert, S. (2008). Vitamin D regulation of cathelicidin in the skin: toward a renaissance of vitamin D in dermatology? *J Invest Dermatol*, 128(4), 773-775. doi:10.1038/jid.2008.35
- Segawa, H., Yamanaka, S., Ohno, Y., Onitsuka, A., Shiozawa, K., Aranami, F., . . . Miyamoto, K. (2007). Correlation between hyperphosphatemia and type II Na-Pi cotransporter activity in klotho mice. *Am J Physiol Renal Physiol*, 292(2), F769-779. doi:10.1152/ajprenal.00248.2006
- Seiki, S., Chonchol, M., Cheung, A. K., Kaufman, J. S., Greene, T., Roberts, W. L., . . . Investigators, H. (2012). 25-hydroxyvitamin D deficiency is associated with an increased risk of metabolic syndrome in patients with non-diabetic chronic kidney disease. *Clin Nephrol*, 78(6), 432-441. doi:10.5414/CN107498
- Seiler, S., Heine, G. H., & Fliser, D. (2009). Clinical relevance of FGF-23 in chronic kidney disease. *Kidney Int*, 76, S34-S42.

- Sepehrmanesh, Z., Kolahdooz, F., Abedi, F., Mazroei, N., Assarian, A., Asemi, Z., & Esmailzadeh, A. (2016). Vitamin D Supplementation Affects the Beck Depression Inventory, Insulin Resistance, and Biomarkers of Oxidative Stress in Patients with Major Depressive Disorder: A Randomized, Controlled Clinical Trial. *J Nutr*, 146(2), 243-248. doi:10.3945/jn.115.218883
- Shaffer, P. L., McDonnell, D. P., & Gewirth, D. T. (2005). Characterization of transcriptional activation and DNA-binding functions in the hinge region of the vitamin D receptor. *Biochemistry*, 44(7), 2678-2685. doi:10.1021/bi0477182
- Shahin, Y., Barakat, H., Barnes, R., & Chetter, I. (2013). The Vicorder device compared with SphygmoCor in the assessment of carotid–femoral pulse wave velocity in patients with peripheral arterial disease. *Hypertension Research*, 36(3), 208-212.
- Shanahan, C. M. (2005). Mechanisms of vascular calcification in renal disease. *Clin Nephrol*, 63(2), 146-157.
- Shanahan, C. M., Crouthamel, M. H., Kapustin, A., & Giachelli, C. M. (2011). Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. *Circ Res*, 109(6), 697-711.
- Sharifi, N., Amani, R., Hajiani, E., & Cheraghian, B. (2014). Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocrine*, 47(1), 70-80. doi:10.1007/s12020-014-0336-5
- Shaw, N. J., & Mughal, M. Z. (2013). Vitamin D and child health part 1 (skeletal aspects). *Arch Dis Child*, 98(5), 363-367. doi:10.1136/archdischild-2011-301264
- Shaw, P. X. (2004). Rethinking oxidized low-density lipoprotein, its role in atherogenesis and the immune responses associated with it. *ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS-ENGLISH EDITION*-, 52(4), 225-239.
- Shearer, M. J. (1997). The roles of vitamins D and K in bone health and osteoporosis prevention. *Proc Nutr Soc*, 56(3), 915-937.
- Shimada, T., Hasegawa, H., Yamazaki, Y., Muto, T., Hino, R., Takeuchi, Y., . . . Yamashita, T. (2004). FGF-23 is a potent regulator of vitamin D metabolism

- and phosphate homeostasis. *J Bone Miner Res*, 19(3), 429-435. doi:10.1359/jbmr.0301264
- Shimada, T., Kakitani, M., Yamazaki, Y., Hasegawa, H., Takeuchi, Y., Fujita, T., . . . Yamashita, T. (2004). Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest*, 113(4), 561-568. doi:10.1172/JCI19081
- Shimada, T., Urakawa, I., Isakova, T., Yamazaki, Y., Epstein, M., Wesseling-Perry, K., . . . Juppner, H. (2010). Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. *J Clin Endocrinol Metab*, 95(2), 578-585. doi:10.1210/jc.2009-1603
- Shinki, T., Ueno, Y., DeLuca, H. F., & Suda, T. (1999). Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D3-1alpha-hydroxylase gene in normocalcemic rats. *Proc Natl Acad Sci U S A*, 96(14), 8253-8258.
- Shoag, J., & Eisner, B. H. (2014). Relationship between C-reactive protein and kidney stone prevalence. *The Journal of urology*, 191(2), 372-375.
- Shroff, R., Speer, T., Colin, S., Charakida, M., Zewinger, S., Staels, B., . . . O'Neill, F. (2014). HDL in children with CKD promotes endothelial dysfunction and an abnormal vascular phenotype. *J Am Soc Nephrol*, 25(11), 2658-2668.
- Silver, A. E., Beske, S. D., Christou, D. D., Donato, A. J., Moreau, K. L., Eskurza, I., . . . Seals, D. R. (2007). Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. *Circulation*, 115(5), 627-637. doi:10.1161/circulationaha.106.657486
- Singh, G., & Bonham, A. J. (2014). A predictive equation to guide vitamin D replacement dose in patients. *J Am Board Fam Med*, 27(4), 495-509. doi:10.3122/jabfm.2014.04.130306
- Slatopolsky, E., & Delmez, J. (1996). Pathogenesis of secondary hyperparathyroidism. *Nephrol Dial Transplant*, 11(suppl3), 130-135.
- Smith, L. H. (1990). Renal stones. Solutions and solute. *Endocrinol Metab Clin North Am*, 19(4), 767-772.
- Smolders, J., Peelen, E., Thewissen, M., Menheere, P., Tervaert, J. W., Hupperts, R., & Damoiseaux, J. (2009). The relevance of vitamin D receptor gene

- polymorphisms for vitamin D research in multiple sclerosis. *Autoimmun Rev*, 8(7), 621-626. doi:10.1016/j.autrev.2009.02.009
- Smolders, J., Peelen, E., Thewissen, M., Menheere, P., Tervaert, J. W. C., Hupperts, R., & Damoiseaux, J. (2009). The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. *Autoimmun Rev*, 8(7), 621-626.
- Snijder, M. B., Lips, P., Seidell, J. C., Visser, M., Deeg, D. J., Dekker, J. M., & van Dam, R. M. (2007). Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med*, 261(6), 558-565. doi:10.1111/j.1365-2796.2007.01778.x
- Snijder, M. B., van Dam, R. M., Visser, M., Deeg, D. J., Dekker, J. M., Bouter, L. M., . . . Lips, P. (2005). Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab*, 90(7), 4119-4123.
- Somjen, D., Weisman, Y., Kohen, F., Gayer, B., Limor, R., Sharon, O., . . . Stern, N. (2005). 25-hydroxyvitamin D3-1 α -hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation*, 111(13), 1666-1671. doi:10.1161/01.cir.0000160353.27927.70
- Song, Y., Peng, X., Porta, A., Takanaga, H., Peng, J. B., Hediger, M. A., . . . Christakos, S. (2003). Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. *Endocrinology*, 144(9), 3885-3894. doi:10.1210/en.2003-0314
- Soro-Paavonen, A., Westerbacka, J., Ehnholm, C., & Taskinen, M. R. (2006). Metabolic syndrome aggravates the increased endothelial activation and low-grade inflammation in subjects with familial low HDL. *Ann Med*, 38(3), 229-238.
- Sorrentino, S. A., Besler, C., Rohrer, L., Meyer, M., Heinrich, K., Bahlmann, F. H., . . . Landmesser, U. (2010). Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. *Circulation*, 121(1), 110-122. doi:10.1161/CIRCULATIONAHA.108.836346

- Speer, T., Rohrer, L., Blyszczuk, P., Shroff, R., Kuschnerus, K., Krankel, N., . . . Landmesser, U. (2013). Abnormal high-density lipoprotein induces endothelial dysfunction via activation of Toll-like receptor-2. *Immunity*, 38(4), 754-768. doi:10.1016/j.immuni.2013.02.009
- Spina, C., Tangpricha, V., Yao, M., Zhou, W., Wolfe, M. M., Maehr, H., . . . Holick, M. F. (2005). Colon cancer and solar ultraviolet B radiation and prevention and treatment of colon cancer in mice with vitamin D and its Gemini analogs. *J Steroid Biochem Mol Biol*, 97(1-2), 111-120. doi:10.1016/j.jsbmb.2005.06.003
- Spiro, A., & Buttriss, J. L. (2014). Vitamin D: An overview of vitamin D status and intake in Europe. *Nutr Bull*, 39(4), 322-350. doi:10.1111/nbu.12108
- Stapleton, A. M., Timme, T. L., & Ryall, R. L. (1998). Gene expression of prothrombin in the human kidney and its potential relevance to kidney stone disease. *Br J Urol*, 81(5), 666-671; discussion 671-662.
- Stewart, A. D., Jiang, B., Millasseau, S. C., Ritter, J. M., & Chowienczyk, P. J. (2006). Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. *Hypertension*, 48(3), 404-410.
- Stewart, A. D., Millasseau, S. C., Kearney, M. T., Ritter, J. M., & Chowienczyk, P. J. (2003). Effects of inhibition of basal nitric oxide synthesis on carotid-femoral pulse wave velocity and augmentation index in humans. *Hypertension*, 42(5), 915-918.
- Stokić, E., Kupusinac, A., Tomić-Naglić, D., Zavišić, B. K., Mitrović, M., Smiljenić, D., . . . Isenović, E. (2015). Obesity and vitamin D deficiency: trends to promote a more proatherogenic cardiometabolic risk profile. *Angiology*, 66(3), 237-243.
- Stoll, F., Akladios, C. Y., & Mathelin, C. (2013). [Vitamin D and breast cancer: is there a link?]. *Gynecol Obstet Fertil*, 41(4), 242-250. doi:10.1016/j.gyobfe.2013.02.002
- Sunbul, M., Cincin, A., Bozbay, M., Mammadov, C., Atas, H., Ozsenel, E. B., . . . Basaran, Y. (2016). Arterial stiffness parameters associated with vitamin D deficiency and supplementation in patients with normal cardiac functions. *Turk Kardiyol Dern Ars*, 44(4), 281-288. doi:10.5543/tkda.2015.93237

- Sung, J. W., Lee, S. H., Byrne, C. D., Chung, P. W., Won, Y. S., & Sung, K. C. (2014). High-sensitivity C-reactive protein is associated with the presence of coronary artery calcium in subjects with normal blood pressure but not in subjects with hypertension. *Arch Med Res*, 45(2), 170-176. doi:10.1016/j.arcmed.2014.01.003
- Szabó, C., Ischiropoulos, H., & Radi, R. (2007). Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov*, 6(8), 662-680.
- Szczygielska, A., Widomska, S., Jaraszkiewicz, M., Knera, P., & Muc, K. (2003). Blood lipids profile in obese or overweight patients. *Ann Univ Mariae Curie Skłodowska Med*, 58(2), 343-349.
- Taha, H., Arya, A., Paydar, M., Looi, C. Y., Wong, W. F., Vasudeva Murthy, C. R., . . . Hadi, A. H. (2014). Upregulation of insulin secretion and downregulation of pro-inflammatory cytokines, oxidative stress and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats by *Pseuduvaria monticola* bark extract. *Food Chem Toxicol*, 66, 295-306. doi:10.1016/j.fct.2014.01.054
- Takahashi, S., Yamamoto, T., Moriwaki, Y., Tsutsumi, Z., Yamakita, J., & Higashino, K. (1998). Decreased serum concentrations of 1,25(OH)₂-vitamin D₃ in patients with gout. *Metabolism*, 47(3), 336-338.
- Takemoto, F., Shinki, T., Yokoyama, K., Inokami, T., Hara, S., Yamada, A., . . . Uchida, S. (2003). Gene expression of vitamin D hydroxylase and megalin in the remnant kidney of nephrectomized rats. *Kidney Int*, 64(2), 414-420. doi:10.1046/j.1523-1755.2003.00114.x
- Takiishi, T., Gysemans, C., Bouillon, R., & Mathieu, C. (2010). Vitamin D and diabetes. *Endocrinol Metab Clin North Am*, 39(2), 419-446, table of contents. doi:10.1016/j.ecl.2010.02.013
- Talmor, Y., Golan, E., Benchetrit, S., Bernheim, J., Klein, O., Green, J., & Rashid, G. (2008). Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. *Am J Physiol Renal Physiol*, 294(5), F1059-1064. doi:10.1152/ajprenal.00051.2008
- Tan, X., Li, Y., & Liu, Y. (2006). Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. *J Am Soc Nephrol*, 17(12), 3382-3393. doi:10.1681/asn.2006050520

- Tan, X., Wen, X., & Liu, Y. (2008). Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF-kappaB signaling. *J Am Soc Nephrol*, 19(9), 1741-1752. doi:10.1681/asn.2007060666
- Tang, J., McFann, K. K., & Chonchol, M. B. (2012). Association between serum 25-hydroxyvitamin D and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988-94. *Nephrol Dial Transplant*, 27(12), 4385-4389. doi:10.1093/ndt/gfs297
- Tangpricha, V., Kelly, A., Stephenson, A., Maguiness, K., Enders, J., Robinson, K. A., . . . Borowitz, D. (2012). An update on the screening, diagnosis, management, and treatment of vitamin D deficiency in individuals with cystic fibrosis: evidence-based recommendations from the Cystic Fibrosis Foundation. *J Clin Endocrinol Metab*, 97(4), 1082-1093. doi:10.1210/jc.2011-3050
- Tarcin, O., Yavuz, D. G., Ozben, B., Telli, A., Ogunc, A. V., Yuksel, M., . . . Akalin, S. (2009). Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab*, 94(10), 4023-4030. doi:10.1210/jc.2008-1212
- Targher, G., Bertolini, L., Scala, L., Cigolini, M., Zenari, L., Falezza, G., & Arcaro, G. (2007). Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis*, 17(7), 517-524. doi:10.1016/j.numecd.2006.04.002
- Teichmann, A., Dutta, P. C., Staffas, A., & Jägerstad, M. (2007). Sterol and vitamin D 2 concentrations in cultivated and wild grown mushrooms: effects of UV irradiation. *LWT-Food Science and Technology*, 40(5), 815-822.
- Tensiomed. (2016). Arteriograph report. Retrieved from <https://www.welenzijn.nl/upload/files/Voorbeeldmeting%20Arteriograph%20.pdf>
- Thacher, T. D., & Clarke, B. L. (2011). Vitamin D insufficiency. *Mayo Clin Proc*, 86(1), 50-60. doi:10.4065/mcp.2010.0567
- Theodoratou, E., Tzoulaki, I., Zgaga, L., & Ioannidis, J. P. (2014). Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ*, 348, g2035.

- Thraillkill, K. M., Jo, C. H., Cockrell, G. E., Moreau, C. S., & Fowlkes, J. L. (2011). Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency? *J Clin Endocrinol Metab*, 96(1), 142-149. doi:10.1210/jc.2010-0980
- Ticinesi, A., Nouvenne, A., Ferraro, P. M., Folesani, G., Lauretani, F., Allegri, F., . . . Meschi, T. (2016). Idiopathic Calcium Nephrolithiasis and Hypovitaminosis D: A Case-control Study. *Urology*, 87, 40-45. doi:10.1016/j.urology.2015.10.009
- Tirosh, A., Shai, I., Tekes-Manova, D., Israeli, E., Pereg, D., Shochat, T., . . . Rudich, A. (2005). Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med*, 353(14), 1454-1462.
- Tohari, A. M., Zhou, X., & Shu, X. (2016). Protection against oxidative stress by vitamin D in cone cells. *Cell Biochem Funct*, 34(2), 82-94. doi:10.1002/cbf.3167
- Toloza, S. M., Cole, D. E., Gladman, D. D., Ibanez, D., & Urowitz, M. B. (2010). Vitamin D insufficiency in a large female SLE cohort. *Lupus*, 19(1), 13-19. doi:10.1177/0961203309345775
- Tomaschitz, A., Pilz, S., Ritz, E., Grammer, T., Drechsler, C., Boehm, B. O., & März, W. (2010). Independent association between 1, 25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin–angiotensin system: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clinica Chimica Acta*, 411(17), 1354-1360.
- Tonelli, M., Bohm, C., Pandeya, S., Gill, J., Levin, A., & Kiberd, B. A. (2001). Cardiac risk factors and the use of cardioprotective medications in patients with chronic renal insufficiency. *Am J Kidney Dis*, 37(3), 484-489.
- Torricelli, F. C., De, S. K., Gebreselassie, S., Li, I., Sarkissian, C., & Monga, M. (2014). Dyslipidemia and kidney stone risk. *J Urol*, 191(3), 667-672. doi:10.1016/j.juro.2013.09.022
- Toubi, E., & Shoenfeld, Y. (2010). The role of vitamin D in regulating immune responses. *Isr Med Assoc J*, 12(3), 174-175.
- Townsend, N., Wilson, L., Bhatnagar, P., Wickramasinghe, K., Rayner, M., & Nichols, M. (2016). Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J*. doi:10.1093/eurheartj/ehw334

- Trinchieri, A. (2014). [Urinary calculi and infection]. *Urologia*, 81(2), 93-98. doi:10.5301/uro.5000073
- Tripepi, G., Mallamaci, F., & Zoccali, C. (2005). Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. *J Am Soc Nephrol*, 16 Suppl 1, S83-88.
- Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., . . . Lanham-New, S. (2012). Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr*, 95(6), 1357-1364. doi:10.3945/ajcn.111.031070
- Trujillo, G., Habel, D. M., Ge, L., Ramadass, M., Cooke, N. E., & Kew, R. R. (2013). Neutrophil recruitment to the lung in both C5a- and CXCL1-induced alveolitis is impaired in vitamin D-binding protein-deficient mice. *J Immunol*, 191(2), 848-856. doi:10.4049/jimmunol.1202941
- Tsiaras, W. G., & Weinstock, M. A. (2011). Factors influencing vitamin D status. *Acta dermato-venereologica*, 91(2), 115-124.
- Tso, P., & Fujimoto, K. (1991). The absorption and transport of lipids by the small intestine. *Brain Res Bull*, 27(3-4), 477-482.
- Tungsanga, K., Sriboonlue, P., Futrakul, P., Yachantha, C., & Tosukhowong, P. (2005). Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. *Urol Res*, 33(1), 65-69.
- Turer, C. B., Lin, H., & Flores, G. (2013). Prevalence of vitamin D deficiency among overweight and obese US children. *Pediatrics*, 131(1), e152-161. doi:10.1542/peds.2012-1711
- Turney, B. W., Appleby, P. N., Reynard, J. M., Noble, J. G., Key, T. J., & Allen, N. E. (2014). Diet and risk of kidney stones in the Oxford cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Epidemiol*, 29(5), 363-369.
- Turney, B. W., Reynard, J. M., Noble, J. G., & Keoghane, S. R. (2012). Trends in urological stone disease. *BJU Int*, 109(7), 1082-1087. doi:10.1111/j.1464-410X.2011.10495.x
- Uberti, F., Lattuada, D., Morsanuto, V., Nava, U., Bolis, G., Vacca, G., . . . Molinari, C. (2014). Vitamin D protects human endothelial cells from oxidative stress

- through the autophagic and survival pathways. *J Clin Endocrinol Metab*, 99(4), 1367-1374. doi:10.1210/jc.2013-2103
- Udayakumar, P., Balasubramanian, S., Ramalingam, K., Lakshmi, C., Srinivas, C., & Mathew, A. C. (2006). Cutaneous manifestations in patients with chronic renal failure on hemodialysis. *Indian J Dermatol, Venereol Leprol*, 72(2), 119.
- Umekawa, T., Chegini, N., & Khan, S. R. (2002). Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. *Kidney Int*, 61(1), 105-112. doi:10.1046/j.1523-1755.2002.00106.x
- Umekawa, T., Chegini, N., & Khan, S. R. (2003). Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals. *Nephrol Dial Transplant*, 18(4), 664-669.
- Upala, S., Sanguankeo, A., Congrete, S., & Jaruvongvanich, V. (2016). Effect of cholecalciferol supplementation on arterial stiffness: a systematic review and meta-analysis. *Scand Cardiovasc J*, 50(4), 230-235. doi:10.3109/14017431.2016.1173226
- Vaidya, A., Sun, B., Larson, C., Forman, J. P., & Williams, J. S. (2012). Vitamin D3 therapy corrects the tissue sensitivity to angiotensin ii akin to the action of a converting enzyme inhibitor in obese hypertensives: an interventional study. *J Clin Endocrinol Metab*, 97(7), 2456-2465.
- Valdivielso, J. M., & Fernandez, E. (2006). Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta*, 371(1-2), 1-12. doi:10.1016/j.cca.2006.02.016
- van Ballegooijen, A. J., Reinders, I., Visser, M., & Brouwer, I. A. (2013). Parathyroid hormone and cardiovascular disease events: A systematic review and meta-analysis of prospective studies. *Am Heart J*, 165(5), 655-664, 664 e651-655. doi:10.1016/j.ahj.2013.02.014
- van Etten, E., & Mathieu, C. (2005). Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol*, 97(1-2), 93-101. doi:10.1016/j.jsbmb.2005.06.002
- Van Gaal, L. F., Mertens, I. L., & De Block, C. E. (2006). Mechanisms linking obesity with cardiovascular disease. *Nature*, 444(7121), 875-880. doi:10.1038/nature05487

- Van Laer, K., Hamilton, C. J., & Messens, J. (2013). Low-molecular-weight thiols in thiol-disulfide exchange. *Antioxid Redox Signal*, 18(13), 1642-1653. doi:10.1089/ars.2012.4964
- van Rooijen, J. J., Voskamp, A. F., Kamerling, J. P., & Vliegenthart, J. F. (1999). Glycosylation sites and site-specific glycosylation in human Tamm-Horsfall glycoprotein. *Glycobiology*, 9(1), 21-30.
- Vanchinathan, V., & Lim, H. W. (2012). A dermatologist's perspective on vitamin D. *Mayo Clin Proc*, 87(4), 372-380. doi:10.1016/j.mayocp.2011.12.010
- Vandevijvere, S., Amsalkhir, S., Van Oyen, H., & Moreno-Reyes, R. (2012). High prevalence of vitamin D deficiency in pregnant women: a national cross-sectional survey. *PLoS One*, 7(8), e43868. doi:10.1371/journal.pone.0043868
- Vanholder, R., Patel, S., & Hsu, C. H. (1993). Effect of uric acid on plasma levels of 1,25(OH)₂D in renal failure. *J Am Soc Nephrol*, 4(4), 1035-1038.
- Vaziri, N. D., & Moradi, H. (2006). Mechanisms of dyslipidemia of chronic renal failure. *Hemodial Int*, 10(1), 1-7. doi:10.1111/j.1542-4758.2006.01168.x
- Vaziri, N. D., & Norris, K. (2011). Lipid disorders and their relevance to outcomes in chronic kidney disease. *Blood Purif*, 31(1-3), 189-196.
- Vega, G. L., Toto, R. D., & Grundy, S. M. (1995). Metabolism of low density lipoproteins in nephrotic dyslipidemia: comparison of hypercholesterolemia alone and combined hyperlipidemia. *Kidney Int*, 47(2), 579-586.
- Verstuyf, A., Carmeliet, G., Bouillon, R., & Mathieu, C. (2010). Vitamin D: a pleiotropic hormone. *Kidney Int*, 78(2), 140-145. doi:10.1038/ki.2010.17
- Vimaleswaran, K. S., Berry, D. J., Lu, C., Tikkanen, E., Pilz, S., Hiraki, L. T., . . . Hypponen, E. (2013). Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*, 10(2), e1001383. doi:10.1371/journal.pmed.1001383
- vinh quoc Lu'o'ng, K., & Nguyen, L. T. (2013). The beneficial role of vitamin D in obesity: possible genetic and cell signaling mechanisms. *Nutr J*, 12, 89. doi:10.1186/1475-2891-12-89
- Viswanathan, P., Rimer, J. D., Kolbach, A. M., Ward, M. D., Kleinman, J. G., & Wesson, J. A. (2011). Calcium oxalate monohydrate aggregation induced by aggregation of desialylated Tamm-Horsfall protein. *Urol Res*, 39(4), 269-282. doi:10.1007/s00240-010-0353-7

- Vogiatzi, G., Tousoulis, D., & Stefanadis, C. (2009). The role of oxidative stress in atherosclerosis. *Hellenic J Cardiol*, 50(5), 402-409.
- Vogt, S., Baumert, J., Peters, A., Thorand, B., & Scragg, R. (2016). Effect of waist circumference on the association between serum 25-hydroxyvitamin D and serum lipids: results from the National Health and Nutrition Examination Survey 2001–2006. *Public Health Nutr*, 1-10.
- von Hurst, P. R., Stonehouse, W., & Coad, J. (2010). Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr*, 103(4), 549-555. doi:10.1017/s0007114509992017
- Vural, M., Camuzcuoglu, H., Toy, H., Cece, H., Aydin, H., Eren, M. A., . . . Aksoy, N. (2012). Evaluation of the future atherosclerotic heart disease with oxidative stress and carotid artery intima media thickness in gestational diabetes mellitus. *Endocr Res*, 37(3), 145-153. doi:10.3109/07435800.2012.654556
- Waldron, J. L., Ashby, H. L., Cornes, M. P., Bechervaise, J., Razavi, C., Thomas, O. L., . . . Gama, R. (2013). Vitamin D: a negative acute phase reactant. *J Clin Pathol*, jclinpath-2012-201301.
- Wallis, D. E., Penckofer, S., & Sizemore, G. W. (2008). The "sunshine deficit" and cardiovascular disease. *Circulation*, 118(14), 1476-1485. doi:10.1161/circulationaha.107.713339
- Walsh, J. S., Evans, A. L., Bowles, S., Naylor, K. E., Jones, K. S., Schoenmakers, I., . . . Eastell, R. (2016). Free 25-hydroxyvitamin D is low in obesity, but there are no adverse associations with bone health. *Am J Clin Nutr*, 103(6), 1465-1471. doi:10.3945/ajcn.115.120139
- Wamberg, L., Christiansen, T., Paulsen, S., Fisker, S., Rask, P., Rejnmark, L., . . . Pedersen, S. B. (2013). Expression of vitamin D-metabolizing enzymes in human adipose tissue—the effect of obesity and diet-induced weight loss. *Int J Obesity*, 37(5), 651-657.
- Wamberg, L., Kampmann, U., Stodkilde-Jorgensen, H., Rejnmark, L., Pedersen, S. B., & Richelsen, B. (2013). Effects of vitamin D supplementation on body fat accumulation, inflammation, and metabolic risk factors in obese adults with low vitamin D levels - results from a randomized trial. *Eur J Intern Med*, 24(7), 644-649. doi:10.1016/j.ejim.2013.03.005

- Wang, H., Xia, N., Yang, Y., & Peng, D. Q. (2012). Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials. *Lipids Health Dis*, 11, 42. doi:10.1186/1476-511x-11-42
- Wang, L., Ma, J., Manson, J. E., Buring, J. E., Gaziano, J. M., & Sesso, H. D. (2013). A prospective study of plasma vitamin D metabolites, vitamin D receptor gene polymorphisms, and risk of hypertension in men. *Eur J Nutr*, 52(7), 1771-1779. doi:10.1007/s00394-012-0480-8
- Wang, L., Song, Y., Manson, J. E., Pilz, S., März, W., Michaëlsson, K., . . . Zhang, C. (2012). Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease a meta-analysis of prospective studies. *Circulation: Cardiovascular Quality and Outcomes*, 5(6), 819-829.
- Wang, M., & Lakatta, E. G. (2002). Altered regulation of matrix metalloproteinase-2 in aortic remodeling during aging. *Hypertension*, 39(4), 865-873.
- Wang, T. J., Pencina, M. J., Booth, S. L., Jacques, P. F., Ingelsson, E., Lanier, K., . . . Vasan, R. S. (2008). Vitamin D deficiency and risk of cardiovascular disease. *Circulation*, 117(4), 503-511. doi:10.1161/circulationaha.107.706127
- Wang, T. J., Zhang, F., Richards, J. B., Kestenbaum, B., van Meurs, J. B., Berry, D., . . . Spector, T. D. (2010). Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*, 376(9736), 180-188. doi:10.1016/S0140-6736(10)60588-0
- Wang, Y., Chen, X., Song, Y., Caballero, B., & Cheskin, L. J. (2008). Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int*, 73(1), 19-33. doi:10.1038/sj.ki.5002586
- Wang, Y., Zhou, J., Minto, A., Hack, B., Alexander, J., Haas, M., . . . Quigg, R. (2006). Altered vitamin D metabolism in type II diabetic mouse glomeruli may provide protection from diabetic nephropathy. *Kidney Int*, 70(5), 882-891.
- Watson, K. E., Abrolat, M. L., Malone, L. L., Hoeg, J. M., Doherty, T., Detrano, R., & Demer, L. L. (1997). Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation*, 96(6), 1755-1760.
- Webb, A. R., & Engelsen, O. (2006). Calculated ultraviolet exposure levels for a healthy vitamin D status. *Photochem Photobiol*, 82(6), 1697-1703.
- Webb, A. R., Kline, L., & Holick, M. F. (1988). Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston

- and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab*, 67(2), 373-378. doi:10.1210/jcem-67-2-373
- Weggemans, R. M., Schaafsma, G., & Kromhout, D. (2009). Towards an adequate intake of vitamin D. An advisory report of the Health Council of the Netherlands. *Eur J Clin Nutr*, 63(12), 1455-1457. doi:10.1038/ejcn.2009.67
- Weishaar, R. E., & Simpson, R. U. (1987). Vitamin D3 and cardiovascular function in rats. *J Clin Invest*, 79(6), 1706-1712. doi:10.1172/jci113010
- Weiss, M. F., Erhard, P., Kader-Attia, F. A., Wu, Y. C., Deoreo, P. B., Araki, A., . . . Monnier, V. M. (2000). Mechanisms for the formation of glycoxidation products in end-stage renal disease. *Kidney Int*, 57(6), 2571-2585. doi:10.1046/j.1523-1755.2000.00117.x
- Welsh, J. (2007). Targets of vitamin D receptor signaling in the mammary gland. *J Bone Miner Res*, 22 Suppl 2, V86-90. doi:10.1359/jbmr.07s204
- Werner, C., Pöss, J., & Böhm, M. (2010). Optimal Antagonism of the Renin-Angiotensin-Aldosterone System. *Drugs*, 70(10), 1215-1230.
- Wesson, J. A., Johnson, R. J., Mazzali, M., Beshensky, A. M., Stietz, S., Giachelli, C., . . . Hughes, J. (2003). Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol*, 14(1), 139-147.
- Weyland, P. G., Grant, W. B., & Howie-Esquivel, J. (2014). Does sufficient evidence exist to support a causal association between vitamin D status and cardiovascular disease risk? An assessment using Hill's criteria for causality. *Nutrients*, 6(9), 3403-3430.
- Widlansky, M. E., Gokce, N., Keaney, J. F., Jr., & Vita, J. A. (2003). The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*, 42(7), 1149-1160.
- Williams, P. F., Caterson, I. D., Cooney, G. J., Zilkens, R. R., & Turtle, J. R. (1990). High affinity insulin binding and insulin receptor-effector coupling: modulation by Ca²⁺. *Cell Calcium*, 11(8), 547-556.
- Willnow, T. E., & Nykjaer, A. (2010). Cellular uptake of steroid carrier proteins--mechanisms and implications. *Mol Cell Endocrinol*, 316(1), 93-102. doi:10.1016/j.mce.2009.07.021
- Winkler, G., Kiss, S., Keszthelyi, L., Sápi, Z., Ory, I., Salamon, F., . . . Speer, G. (2003). Expression of tumor necrosis factor (TNF)-alpha protein in the

- subcutaneous and visceral adipose tissue in correlation with adipocyte cell volume, serum TNF-alpha, soluble serum TNF-receptor-2 concentrations and C-peptide level. *Eur J Endocrinol*, 149(2), 129-135.
- Winters, S. J., Chennubhatla, R., Wang, C., & Miller, J. J. (2009). Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women. *Metabolism*, 58(4), 438-442.
- Witham, M., Adams, F., McSwiggan, S., Kennedy, G., Kabir, G., Belch, J., & Khan, F. (2015). Effect of intermittent vitamin D3 on vascular function and symptoms in chronic fatigue syndrome—A randomised controlled trial. *Nutr Metab Cardiovasc Dis*, 25(3), 287-294.
- Witham, M. D., Dove, F. J., Dryburgh, M., Sugden, J. A., Morris, A. D., & Struthers, A. D. (2010). The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia*, 53(10), 2112-2119. doi:10.1007/s00125-010-1838-1
- Witham, M. D., Dove, F. J., Sugden, J. A., Doney, A. S., & Struthers, A. D. (2012). The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis*, 22(10), 864-870. doi:10.1016/j.numecd.2010.11.001
- Witham, M. D., Nadir, M. A., & Struthers, A. D. (2009). Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens*, 27(10), 1948-1954. doi:10.1097/HJH.0b013e32832f075b
- Witham, M. D., Price, R. J., Struthers, A. D., Donnan, P. T., Messow, C. M., Ford, I., & McMurdo, M. E. (2013). Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. *JAMA Intern Med*, 173(18), 1672-1679. doi:10.1001/jamainternmed.2013.9043
- Wolf, M., Molnar, M. Z., Amaral, A. P., Czira, M. E., Rudas, A., Ujszaszi, A., . . . Lakatos, P. (2011). Elevated fibroblast growth factor 23 is a risk factor for kidney transplant loss and mortality. *J Am Soc Nephrol*, 22(5), 956-966.
- Wong, B. W., Meredith, A., Lin, D., & McManus, B. M. (2012). The biological role of inflammation in atherosclerosis. *Can J Cardiol*, 28(6), 631-641. doi:10.1016/j.cjca.2012.06.023
- Wong, M. S., Delansorne, R., Man, R. Y., & Vanhoutte, P. M. (2008). Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of

- the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol*, 295(1), H289-H296.
- Worcester, E. M., Sebastian, J. L., Hiatt, J. G., Beshensky, A. M., & Sadowski, J. A. (1993). The effect of warfarin on urine calcium oxalate crystal growth inhibition and urinary excretion of calcium and nephrocalcin. *Calcif Tissue Int*, 53(4), 242-248.
- Wu, J., Garami, M., Cao, L., Li, Q., & Gardner, D. G. (1995). 1,25(OH)₂D₃ suppresses expression and secretion of atrial natriuretic peptide from cardiac myocytes. *Am J Physiol*, 268(6 Pt 1), E1108-1113.
- Wu, J., Garami, M., Cheng, T., & Gardner, D. G. (1996). 1,25(OH)₂ vitamin D₃ and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes. *J Clin Invest*, 97(7), 1577-1588. doi:10.1172/JCI118582
- Xiang, W., Kong, J., Chen, S., Cao, L.-P., Qiao, G., Zheng, W., . . . Li, Y. C. (2005). Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Ame J Physiol-Endocrinol Metab*, 288(1), E125-E132.
- Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., . . . Tartaglia, L. A. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*, 112(12), 1821-1830.
- Xu, H., Zisman, A. L., Coe, F. L., & Worcester, E. M. (2013). Kidney stones: an update on current pharmacological management and future directions. *Expert opinion on pharmacotherapy*, 14(4), 435-447.
- Yadav, A. K., Banerjee, D., Lal, A., & Jha, V. (2012). Vitamin D deficiency, CD4+CD28null cells and accelerated atherosclerosis in chronic kidney disease. *Nephrology (Carlton)*, 17(6), 575-581. doi:10.1111/j.1440-1797.2012.01611.x
- Yamamoto, S., Yancey, P. G., Ikizler, T. A., Jerome, W. G., Kaseda, R., Cox, B., . . . Kon, V. (2012). Dysfunctional high-density lipoprotein in patients on chronic hemodialysis. *J Am Coll Cardiol*, 60(23), 2372-2379. doi:10.1016/j.jacc.2012.09.013
- Yamate, T., Kohri, K., Umekawa, T., Iguchi, M., & Kurita, T. (1998). Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin-Darby canine kidney cell. *J Urol*, 160(4), 1506-1512.

- Yap, C., Cheung, N. W., Gunton, J. E., Athayde, N., Munns, C. F., Duke, A., & McLean, M. (2014). Vitamin D supplementation and the effects on glucose metabolism during pregnancy: a randomized controlled trial. *Diabetes Care*, 37(7), 1837-1844.
- Yetley, E. A. (2008). Assessing the vitamin D status of the US population. *Am J Clin Nutr*, 88(2), 558S-564S.
- Yim, S., Dhawan, P., Ragunath, C., Christakos, S., & Diamond, G. (2007). Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). *J Cyst Fibros*, 6(6), 403-410. doi:10.1016/j.jcf.2007.03.003
- Yin, X., Yan, L., Lu, Y., Jiang, Q., Pu, Y., & Sun, Q. (2016). Correction of hypovitaminosis D does not improve the metabolic syndrome risk profile in a Chinese population: a randomized controlled trial for 1 year. *Asia Pac J Clin Nutr*, 25(1), 71-77. doi:10.6133/apjcn.2016.25.1.06
- Yiu, A. J., Callaghan, D., Sultana, R., & Bandyopadhyay, B. C. (2015). Vascular Calcification and Stone Disease: A New Look towards the Mechanism. *J Cardiovasc Dev Dis*, 2(3), 141-164. doi:10.3390/jcdd2030141
- Yiu, Y. F., Yiu, K. H., Siu, C. W., Chan, Y. H., Li, S. W., Wong, L. Y., . . . Tse, H. F. (2013). Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. *Atherosclerosis*, 227(1), 140-146. doi:10.1016/j.atherosclerosis.2012.12.013
- Yoon, J. H., Park, D. K., Yong, S. W., & Hong, J. M. (2015). Vitamin D deficiency and its relationship with endothelial dysfunction in patients with early Parkinson's disease. *J Neural Transm (Vienna)*, 122(12), 1685-1691. doi:10.1007/s00702-015-1452-y
- Yousefzadeh, P., Shapses, S. A., & Wang, X. (2014). Vitamin D binding protein impact on 25-hydroxyvitamin D levels under different physiologic and pathologic conditions. *Int J Endocrinol*, 2014.
- Yu, P. J., Skolnick, A., Ferrari, G., Heretis, K., Mignatti, P., Pintucci, G., . . . Grau, J. B. (2009). Correlation between plasma osteopontin levels and aortic valve calcification: potential insights into the pathogenesis of aortic valve calcification and stenosis. *J Thorac Cardiovasc Surg*, 138(1), 196-199. doi:10.1016/j.jtcvs.2008.10.045

- Zahnow, C. A. (2002). CCAAT/enhancer binding proteins in normal mammary development and breast cancer. *Breast Cancer Res*, 4(3), 113-121.
- Zaleski, A., Panza, G., Swales, H., Arora, P., Newton-Cheh, C., Wang, T., . . . Taylor, B. (2015). High-Dose versus Low-Dose Vitamin D Supplementation and Arterial Stiffness among Individuals with Prehypertension and Vitamin D Deficiency. *Dis Markers*, 2015, 918968. doi:10.1155/2015/918968
- Zanni, M. V., Stanley, T. L., Makimura, H., Chen, C. Y., & Grinspoon, S. K. (2010). Effects of TNF-alpha antagonism on E-selectin in obese subjects with metabolic dysregulation. *Clin Endocrinol (Oxf)*, 73(1), 48-54.
- Zehnder, D., Bland, R., Chana, R. S., Wheeler, D. C., Howie, A. J., Williams, M. C., . . . Hewison, M. (2002). Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephrol*, 13(3), 621-629.
- Zehnder, D., Quinkler, M., Eardley, K. S., Bland, R., Lepenies, J., Hughes, S. V., . . . Hewison, M. (2008). Reduction of the vitamin D hormonal system in kidney disease is associated with increased renal inflammation. *Kidney Int*, 74(10), 1343-1353. doi:10.1038/ki.2008.453
- Zella, L. A., Kim, S., Shevde, N. K., & Pike, J. W. (2006). Enhancers located within two introns of the vitamin D receptor gene mediate transcriptional autoregulation by 1,25-dihydroxyvitamin D3. *Mol Endocrinol*, 20(6), 1231-1247. doi:10.1210/me.2006-0015
- Zella, L. A., Meyer, M. B., Nerenz, R. D., Lee, S. M., Martowicz, M. L., & Pike, J. W. (2010). Multifunctional enhancers regulate mouse and human vitamin D receptor gene transcription. *Mol Endocrinol*, 24(1), 128-147. doi:10.1210/me.2009-0140
- Zemel, M. B., Shi, H., Greer, B., Dirienzo, D., & Zemel, P. C. (2000). Regulation of adiposity by dietary calcium. *Faseb j*, 14(9), 1132-1138.
- Zhang, H. Q., Teng, J. H., Li, Y., Li, X. X., He, Y. H., He, X., & Sun, C. H. (2014). Vitamin D status and its association with adiposity and oxidative stress in schoolchildren. *Nutrition*, 30(9), 1040-1044. doi:10.1016/j.nut.2014.02.024
- Zhang, Q.-y., Jiang, C.-m., Sun, C., Tang, T.-f., Jin, B., Cao, D.-w., . . . Zhang, M. (2015). Hypovitaminosis D is associated with endothelial dysfunction in patients with non-dialysis chronic kidney disease. *J Nephrol*, 28(4), 471-476.

- Zhang, Y., Leung, D. Y., Richers, B. N., Liu, Y., Remigio, L. K., Riches, D. W., & Goleva, E. (2012). Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol*, 188(5), 2127-2135. doi:10.4049/jimmunol.1102412
- Zhao, G., Ford, E. S., & Li, C. (2010). Associations of serum concentrations of 25-hydroxyvitamin D and parathyroid hormone with surrogate markers of insulin resistance among U.S. adults without physician-diagnosed diabetes: NHANES, 2003-2006. *Diabetes Care*, 33(2), 344-347. doi:10.2337/dc09-0924
- Zheng, L., Nukuna, B., Brennan, M. L., Sun, M., Goormastic, M., Settle, M., . . . Hazen, S. L. (2004). Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest*, 114(4), 529-541. doi:10.1172/JCI21109
- Zhong, Y., Armbricht, H. J., & Christakos, S. (2009). Calcitonin, a regulator of the 25-hydroxyvitamin D3 1alpha-hydroxylase gene. *J Biol Chem*, 284(17), 11059-11069. doi:10.1074/jbc.M806561200
- Zhou, C., Lu, F., Cao, K., Xu, D., Goltzman, D., & Miao, D. (2008). Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int*, 74(2), 170-179. doi:10.1038/ki.2008.101
- Zhou, H., Xu, C., & Gu, M. (2009). Vitamin D receptor (VDR) gene polymorphisms and Graves' disease: a meta-analysis. *Clin Endocrinol (Oxf)*, 70(6), 938-945. doi:10.1111/j.1365-2265.2008.03413.x
- Zhou, S. F. (2008). Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Curr Drug Metab*, 9(4), 310-322.
- Zieman, S. J., Melenovsky, V., & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol*, 25(5), 932-943. doi:10.1161/01.atv.0000160548.78317.29
- Zierold, C., Mings, J. A., & DeLuca, H. F. (2003). Regulation of 25-hydroxyvitamin D3-24-hydroxylase mRNA by 1,25-dihydroxyvitamin D3 and parathyroid hormone. *J Cell Biochem*, 88(2), 234-237. doi:10.1002/jcb.10341
- Zierold, C., Nehring, J. A., & DeLuca, H. F. (2007). Nuclear receptor 4A2 and C/EBPbeta regulate the parathyroid hormone-mediated transcriptional

- regulation of the 25-hydroxyvitamin D3-1 α -hydroxylase. *Arch Biochem Biophys*, 460(2), 233-239. doi:10.1016/j.abb.2006.11.028
- Zisman, A. L., Evan, A. P., Coe, F. L., & Worcester, E. M. (2015). Do kidney stone formers have a kidney disease? *Kidney Int*, 88(6), 1240-1249.
- Zisman, A. L., Evan, A. P., Coe, F. L., & Worcester, E. M. (2015). Do kidney stone formers have a kidney disease? *Kidney Int*, 88(6), 1240-1249. doi:10.1038/ki.2015.254
- Zisman, A. L., & Wolf, M. (2010). Recent advances in the rapidly evolving field of fibroblast growth factor 23 in chronic kidney disease. *Curr Opin Nephrol Hypertens*, 19(4), 335-342.
- Zitman-Gal, T., Golan, E., Green, J., Bernheim, J., & Benchetrit, S. (2012). Vitamin D receptor activation in a diabetic-like environment: potential role in the activity of the endothelial pro-inflammatory and thioredoxin pathways. *J Steroid Biochem Mol Biol*, 132(1), 1-7.
- Zittermann, A. (2003). Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr*, 89(5), 552-572. doi:10.1079/BJN2003837
- Zittermann, A., Frisch, S., Berthold, H. K., Gotting, C., Kuhn, J., Kleesiek, K., . . . Koerfer, R. (2009). Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am J Clin Nutr*, 89(5), 1321-1327. doi:10.3945/ajcn.2008.27004
- Zittermann, A., & Gummert, J. F. (2010). Nonclassical vitamin D action. *Nutrients*, 2(4), 408-425. doi:10.3390/nu2040408
- Zittermann, A., Iodice, S., Pilz, S., Grant, W. B., Bagnardi, V., & Gandini, S. (2012). Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr*, 95(1), 91-100.
- Zittermann, A., & Koerfer, R. (2008). Vitamin D in the prevention and treatment of coronary heart disease. *Curr Opin Clin Nutr Metab Care*, 11(6), 752-757. doi:10.1097/MCO.0b013e328312c33f
- Zuk, A., Fitzpatrick, T., & Rosella, L. C. (2016). Effect of Vitamin D 3 Supplementation on Inflammatory Markers and Glycemic Measures among Overweight or Obese Adults: A Systematic Review of Randomized Controlled Trials. *PLoS One*, 11(4), e0154215.

APPENDIX 1



***Faculty of Life Sciences
Research Ethics Committee***

frec@chester.ac.uk

Agbalalah Tarimoboere
91a Garden Lane
Chester
CH1 4EW

21st November 2013

Dear Tarimoboere,

Study title: Effect of Vitamin D Supplementation on Cardiometabolic Risk Factors in a Cohort of Overweight and Obese Adult Males in the UK.
FREC reference: 855/13/AT/CSN
Version number: 1

Thank you for sending your application to the Faculty of Life Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The Committee would like to make the following recommendation:-

- On the Three Day Food Diary, amend the title of the 24 Hour Record to 'Example 24 Hour Record' and amend the example list following discussion with your supervisor.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	September 2013
Appendix 1 – List of References	1	September 2013
Appendix 2 – C.V. for Lead Researcher	1	September 2013
Appendix 3 – Letter of Invitation to Participants	1	September 2013
Appendix 4 – Email to Participants	1	September 2013
Appendix 5 – Letter for Permission to Distribute Posters	1	September 2013
Appendix 6 – Recruitment Advertisement	1	September 2013
Appendix 7 – Participant Consent Form	1	September 2013
Appendix 8 – Participant Information Sheet	1	September 2013
Appendix 9 – Venepuncture Risk Assessment	1	September 2013
Appendix 10 – Protocol for Venepuncture	1	September 2013
Appendix 11 – Screening Test Questionnaire	1	September 2013
Appendix 12 – Arterial Stiffness Measurement Protocol	1	September 2013
Appendix 13 – Arterial Stiffness Protocol: Data Collection	1	September 2013
Appendix 14 – Inclusion/Exclusion Criteria	1	September 2013
Appendix 15 – 24 Hour Diet Recall	1	September 2013
Appendix 16 – International Physical Activity Questionnaire	1	September 2013
Appendix 17 – Flow Chart of Study Method	1	September 2013
Response to FREC request for further information or clarification		November 2013
Appendix 6 – Recruitment Poster	2	November 2013
Appendix 7 – Participant Consent Form	2	November 2013
Appendix 8 – Participant Information Sheet	2	November 2013
Appendix 11 – Screening Test Questionnaire	2	November 2013
Appendix 14 – Table of Inclusion and Exclusion Criteria	2	November 2013
Appendix 15 – Three Day Food Diary	2	November 2013
Response to FREC request for further information or clarification		November 2013
Appendix 8 – Participant Information Sheet	3	November 2013
Appendix 11 – Screening Test Questionnaire	3	November 2013
Appendix 15 – Three Day Food Diary	3	November 2013

Please note that this approval is given in accordance with the requirements of English law only. For research taking place wholly or partly within other jurisdictions (including Wales, Scotland and Northern Ireland), you should seek further advice from the Committee Chair / Secretary or the Research and Knowledge Transfer Office and may need additional approval from the appropriate agencies in the country (or countries) in which the research will take place.

With the Committee's best wishes for the success of this project.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'S. Fallows', with a horizontal line underneath.

Dr. Stephen Fallows

Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

Cc. Supervisor/FREC Representative

APPENDIX 2

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts ^{21 31})	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	

Section/Topic	Item No	Checklist item	Reported on page No
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	

Section/Topic	Item No	Checklist item	Reported on page No
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	

Section/Topic	Item No	Checklist item	Reported on page No
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	

Section/Topic	Item No	Checklist item	Reported on page No
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms ²⁸)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	

Section/Topic	Item No	Checklist item	Reported on page No
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

VITAMIN D AND HEALTH STUDY

Vitamin D has long been recognised for its many benefits in relation to maintenance of bone health. However, it may also play a key role in the prevention of heart disease and diabetes. We are carrying out a study to investigate this further and we are looking for male volunteers.

Volunteers will receive a £25 Amazon voucher on completion of study and a free vitamin D test and dietary analysis. We are looking for men aged between 18 and 65 with a BMI above 25kg/m², to take part in a study to investigate the effects of vitamin D supplementation on cardiovascular disease and diabetes risk.

**If you fit this description,
I am interested in hearing from you.**



For more information contact:
Tari Agbalalah
Email: vitaminDstudy@chester.ac.uk



University of
Chester

APPENDIX 4



Participant information sheet

EFFECT OF VITAMIN D SUPPLEMENTATION ON CARDIOMETABOLIC RISK FACTORS IN A COHORT OF OVERWEIGHT AND OBESE ADULT MALES IN THE UK

You are invited to take part in a research study. Before you decide, it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The project is aimed at investigating whether supplementing the diet with oral vitamin D (1 small tablet per day) at a dose of 5000IU/d (125µg) over 8 weeks, will increase vitamin D status, and improve cardiometabolic risk markers such as: blood pressure, inflammation and cholesterol levels.

Why have I been chosen?

You have been chosen because you satisfy the inclusion criteria for the study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

What will happen to me if I take part?

You will attend four clinic sessions on four separate days and also you will be expected to fast (abstain from food, caffeine, alcohol and cigarettes for at least eight hours prior to each clinic day).

Screening Session: Participants will complete a screening questionnaire after which weight, height will be measured. 1mL of blood will then be collected for vitamin D status screening to see if you are eligible for the study. Eligible participants who are vitamin D insufficient will be randomly assigned to receive either vitamin D supplements or placebo (supplements without the active ingredient). You will be asked to complete a three - day food diary and physical activity questionnaire.

Clinic 1 (week 0): Weight, height, waist circumference and blood pressure measurements will be performed. 15mL of blood will then be collected to assess the cardiovascular risk markers.

Clinic 2 (week 4): The procedure performed at clinic 1 will be repeated in this clinic.

Clinic 3 (Week 8): The procedure performed at clinic 2 will be repeated in this clinic.

Each clinic will last approximately 30 minutes.

What are the possible disadvantages and risks of taking part?

There is little or no risk involved in taking part in the study except minor discomfort during blood-taking and taking the supplements.

What are the possible benefits of taking part?

By taking part, you will be contributing to the advancement of knowledge in the role of vitamin D in the prevention of cardiovascular disease. Moreover, other benefits include free vitamin D status test and free dietary analysis and a £25 Amazon/Tesco voucher on completion of the study.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Life Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information.

What will happen to the results of the research study?

The results will be written up into a dissertation for my PhD thesis. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising the research?

The research is conducted as part of a PhD research project in Human Nutrition within the Department of Clinical Sciences and Nutrition at the University of Chester. The study is organised with supervision from the department, by Tari Agbalalah.

Who may I contact for further information?

For more information please contact:

Tari Agbalalah

vitaminDstudy@chester.ac.uk

Thank you for your interest in this research.

APPENDIX 5



CONSENT FORM TO PARTAKE IN STUDY

Title of Project: Effect of vitamin D supplementation on cardiometabolic risk factors in a cohort of overweight and obese adult males in the UK

Name of Researcher: Tari Agbalalah

Please tick box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected.
3. I agree to take part in the above study.

☐☐☐

Name of Participant

Date

Signature

Researcher

Date

Signature

1 for participant; 1 for researcher

APPENDIX 6



SCREENING TEST QUESTIONNAIRE

EFFECT OF VITAMIN D SUPPLEMENTATION ON CARDIOMETABOLIC RISK FACTORS IN A COHORT OF OVERWEIGHT AND OBESE ADULT MALES IN THE UK

Name of participant:
ID:
Email address:
Contact number:
Test date:
DOB:
Ethnicity:

For the reliability and validity of this research study, it is highly relevant that each participant be screened for potential confounders or factors that may affect the results negatively.

Section One

Do you take medications for blood pressure?

YES/NO

Do you take medications for diabetes?

YES/NO

reduction programme

Are you currently partaking in a weight-

YES/NO

Are you taking medication for/ or are you being treated for the following conditions: Bone, kidney, liver or gastrointestinal disease?

YES/NO

Section Two (further information)

Do you take any nutritional supplements? YES/NO

If yes please list:

.....

Are you currently on any other regular medications? YES/NO

If yes please list:

.....

Do you smoke? YES/NO

If yes, how many cigarettes per day on average?

Do you drink alcohol? YES/NO

If yes, how many units of alcohol on average do you consume per week? 1-10 ☐ 11-20 ☐

21-30 ☐ 30+ ☐

APPENDIX 7



Vitamin D Study 3 Day Diet Diary



We would like you to keep his diary of everything you eat and drink over 3 consecutive days. Please do not change your habits because you are keeping this record.

Instructions

- ◆ Record your eating & drinking as you go, not from memory. Remember to include foods/drinks consumed between meals or at night.
- ◆ Ensure you write the day, time, description of meal or drink, amount and preparation method as well as brand of product or recipe as appropriate.
- ◆ Homemade dishes: Record the name of the recipe, ingredients with the amounts & cooking method. These can be recorded on the page at the back of the diary. Take-aways/restaurant: Including as much information as you can eg; vegetable curry with chickpeas, onions, aubergine.
- ◆ Please ensure you record the portion size of the food you eat—there is a guide overleaf to aid you with this.
- ◆ Remember to include any added sugar in drinks/food, salt during cooking or at table and table sauces.
- ◆ Ensure you note details of cooking method—grilled, fried (in oil/butter?), steamed, boiled etc.
- ◆ If foods come with added vitamins or minerals please write this down.
- ◆ Do write down any exercise participated in during each day. Include the nature of exercise (eg swimming) and length of time.
- ◆ Make a note in the margin if there is any wastage and the quantity.

Use the example below to assist you with completing your food diary

Day: Monday

Date: 21st October

<u>Time</u>	<u>Location</u>	<u>Description of food/drink consumed</u>	<u>Brand</u>	<u>Amount/ Portion Size</u>
7.50am	Home	Bran flakes with semi skimmed milk. Banana	Tesco Bran Flakes.	1 cup cereal, 1/2 cup milk 1/2 banana
12.30pm	Work	Sandwich Tuna Light mayo Tomato Flapjack	Hovis wholemeal, Tesco tuna Tesco mayo, Homemade flapjack (See recipe, serves 15)	2 medium slices. 1 can tuna 2 tbsp. mayo 1 tomato 2 servings flapjack

Guide to Portion Sizes:

For foods, quantity can be described using:

Household measures: eg 1 tsp of sugar, 2 thick slices of bread, 1/2 cup gravy.

Weights from labels: eg 125g pot yoghurt, 420g tin baked beans

Number of items: 4 fish fingers, 1 regular sized doughnut

For drinks, quantity can be described using:

The size of the glass (eg large glass) or the volume (300ml)

Day:

Date:

<u>Time</u>	<u>Location</u>	<u>Description of food / drink consumed</u>	<u>Brand</u>	<u>Amount/Quantity</u>

Recipes

For any homemade meals/snacks please write down the ingredients used, quantities and preparation method in the space provided.

[illegible]

APPENDIX 8

PROTOCOL FOR VENEPUNCTURE

Objectives of venepuncture:

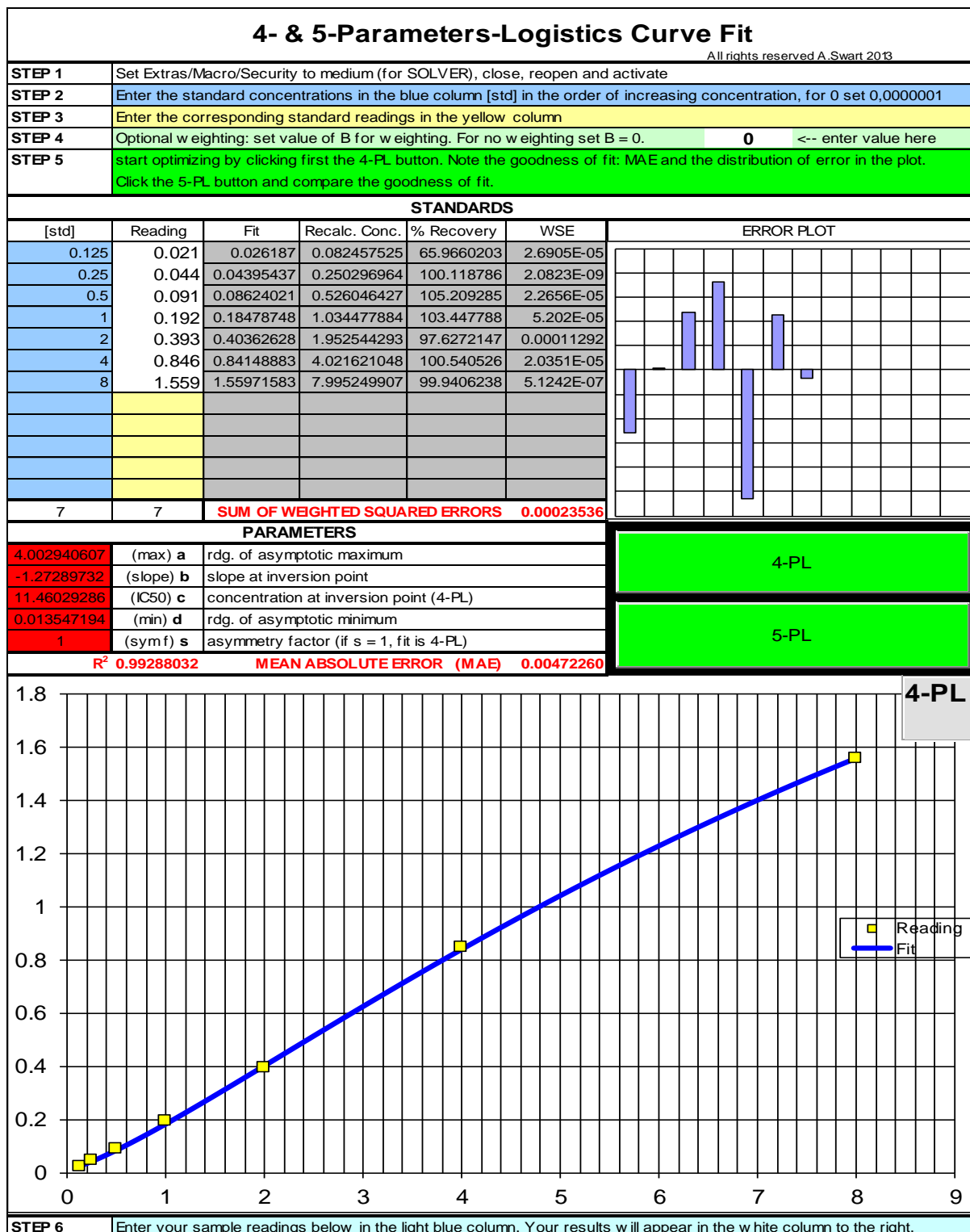
- To obtain a blood sample of adequate volume for the test requested.
- Avoiding pre-analytical interference
- Into the correct tubes for analysis
- Safely
- With minimum discomfort to patient

Procedure:

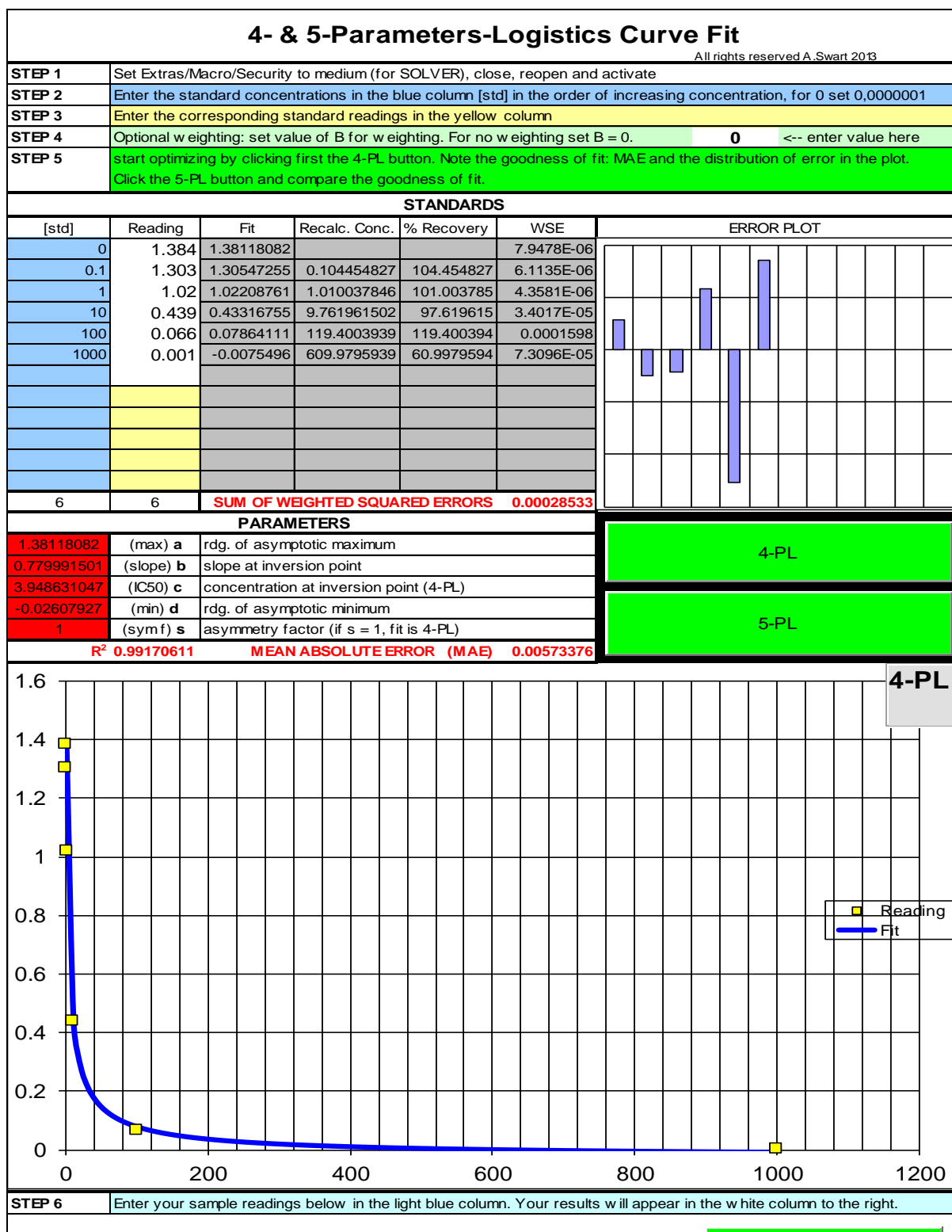
1. Approach patient and explain the procedure, and thereafter, allow patient to ask questions and discuss any problems which have arisen previously.
2. Identify patient, making sure the information matches the details on the requisition form.
3. Reassure patient that the minimum amount of blood required for testing will be drawn
4. Assemble the necessary equipment
5. Wash hands or use sanitizer, allow to dry before wearing gloves
6. Position patient with the chosen arm extended to form a straight- line from shoulder to wrist, with the patient assisting by clenching and unclenching the fist.
7. Select appropriate vein for venepuncture and do not attempt a venepuncture more than twice.
8. Apply the tourniquet 3-4 inches above the collection site, if a tourniquet is used for preliminary vein selection, release it and reapply after two minutes and never leave tourniquet on for over one minute. Clean puncture site by making a smooth circular pass over the site with the 70% alcohol pad, allow to dry. Do not repalpate the insertion site.
9. Anchor vein by applying manual traction and the insert needle smoothly at an angle of 20-30°.
10. Holding the hub securely, insert the first vacutainer tube following proper order of draw into the large end of the hub penetrating the stopper. Blood should flow into the evacuated tube, after blood starts to flow release the tourniquet and ask patient to unclench hand.
11. When blood stops flowing, remove the tube by holding hub securely and pulling the tube off the needle.
12. If multiple tubes are needed, the proper order of draw to avoid cross contamination and erroneous results is as follows:
13. Blood culture vials or bottles, sterile tubes
14. Coagulation tube (light blue top)
15. Serum tube with or without clot activator or silica gel (Red or Gold)
16. Heparin tube (Green top)
17. EDTA (lavender top)
18. Glycolytic inhibitor
19. Each coagulation tube (light blue top) should be gently inverted 4 times after being removed from the hub. Red and gold tops should be inverted five times from the hub. All other additive containing tubes should be gently inverted 8-10 times.

20. Place a gauze pad over the puncture site and remove the needle, and immediately apply slight pressure .ask the patient to apply pressure for at least 2 minutes, when bleeding stops, apply a fresh bandage, gauze or tape.
21. Properly dispose of hub with needle attached into a sharps bin- label all tubes with patient labels, initials, date and time.

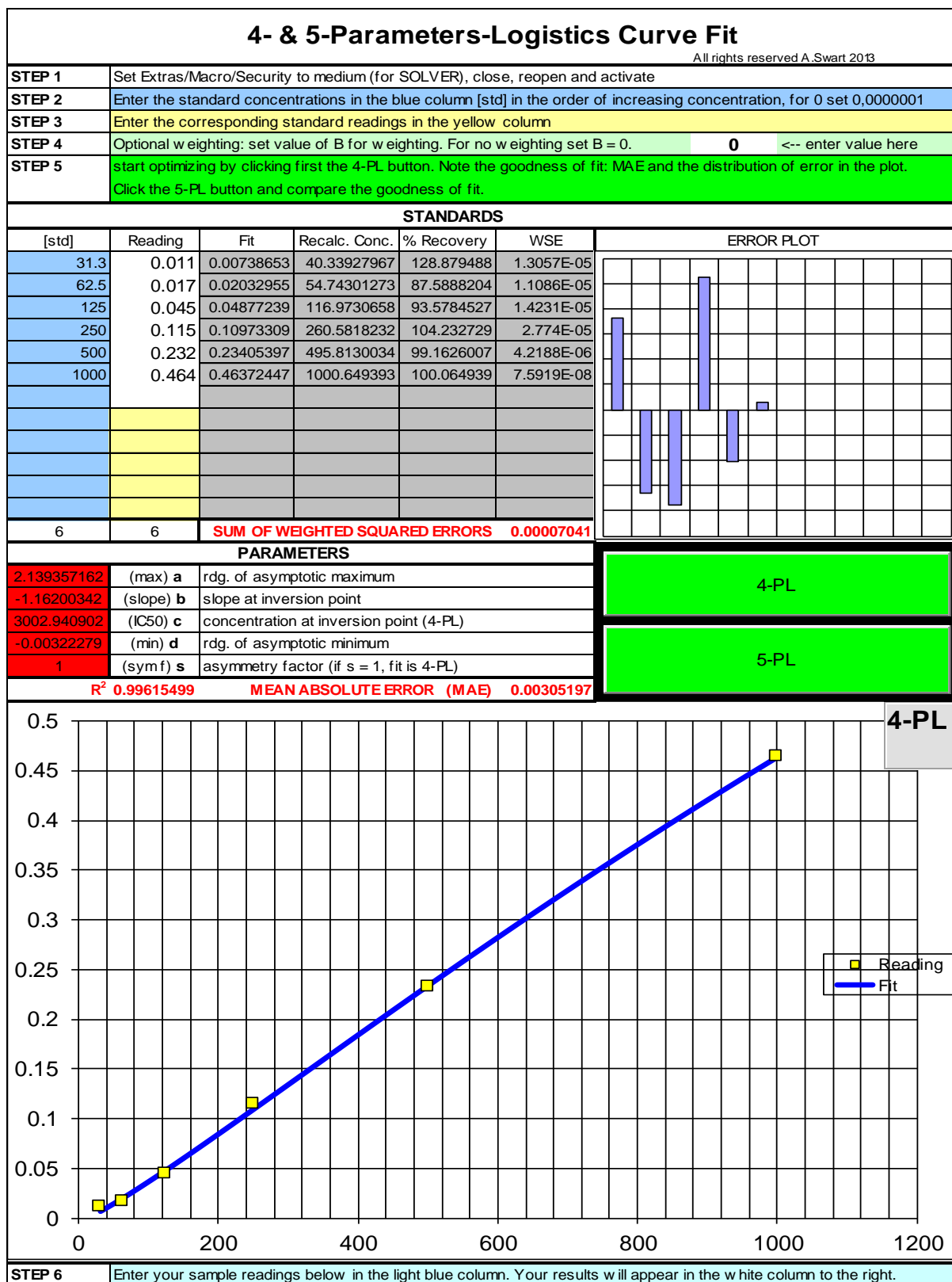
APPENDIX 9



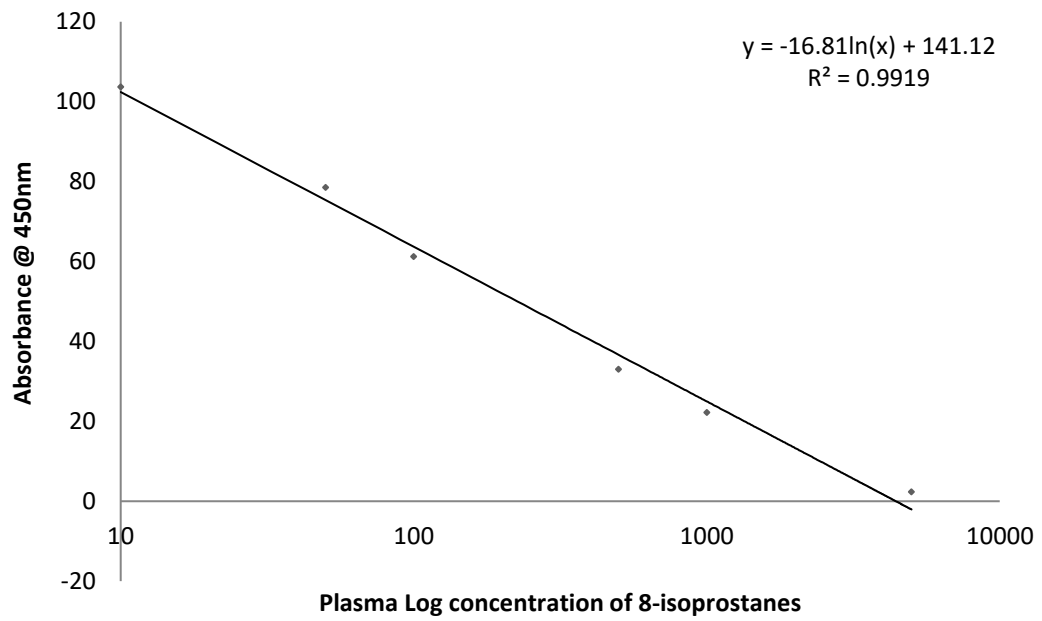
Example calibration curve obtained for plasma sE-selectin concentration



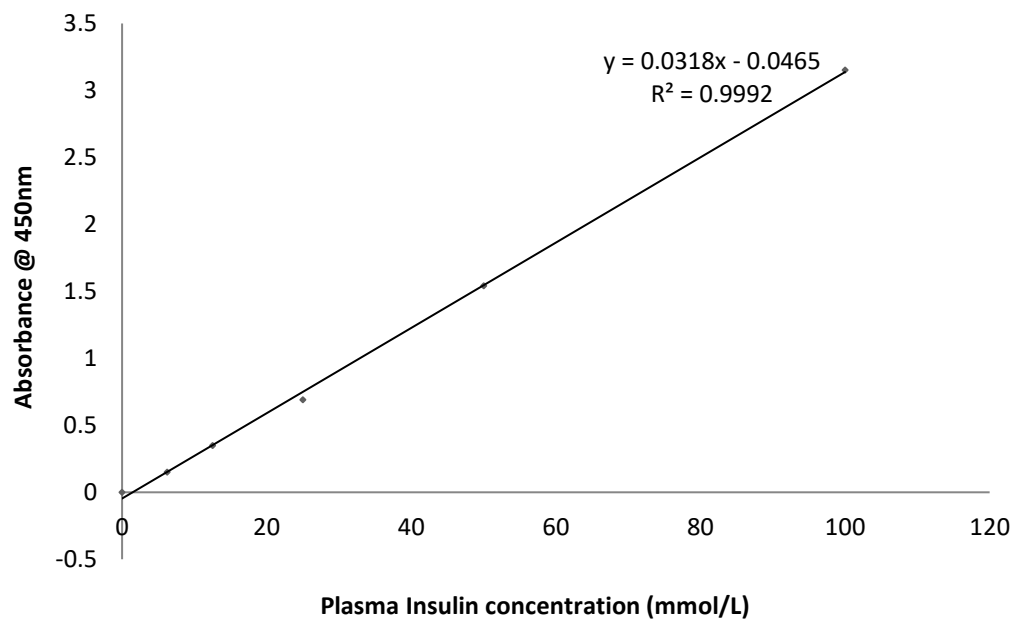
Example calibration curve obtained for plasma angiotensin II concentration



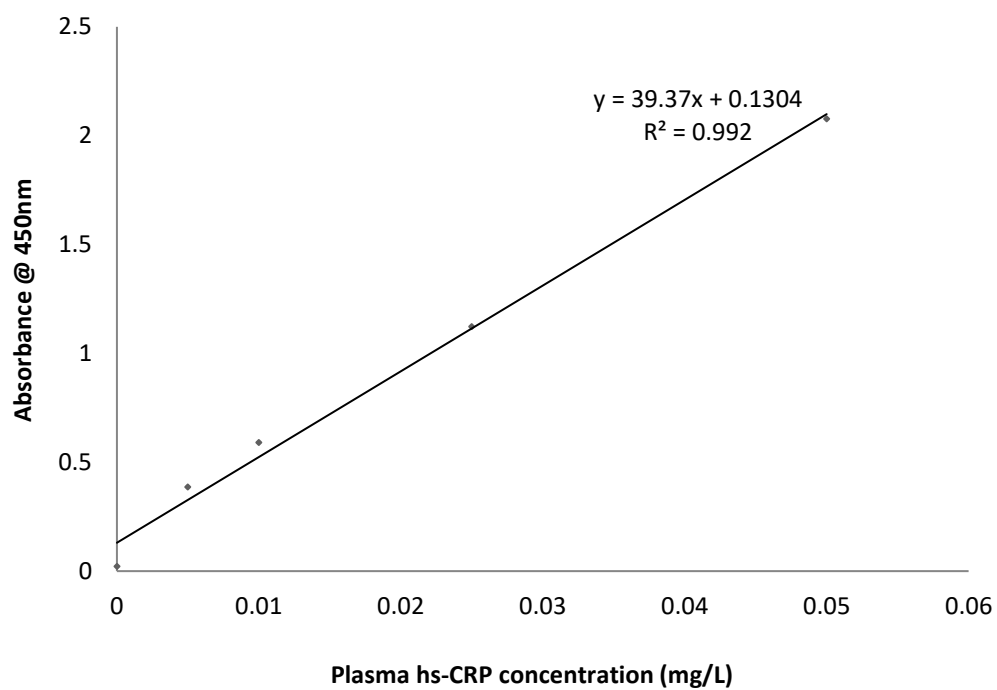
Example calibration curve obtained for plasma renin concentration



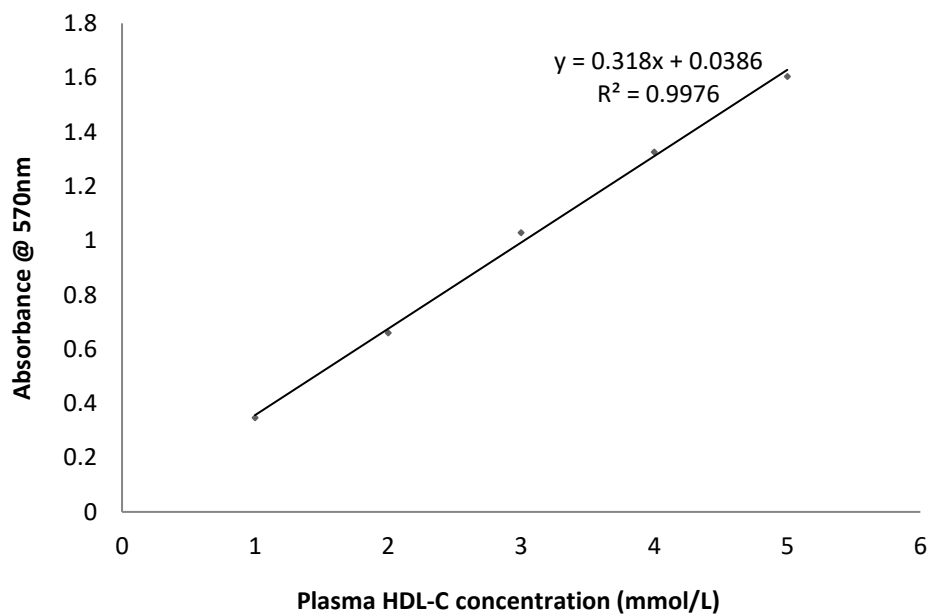
Example calibration curve obtained for plasma 8-isoprostanes concentration



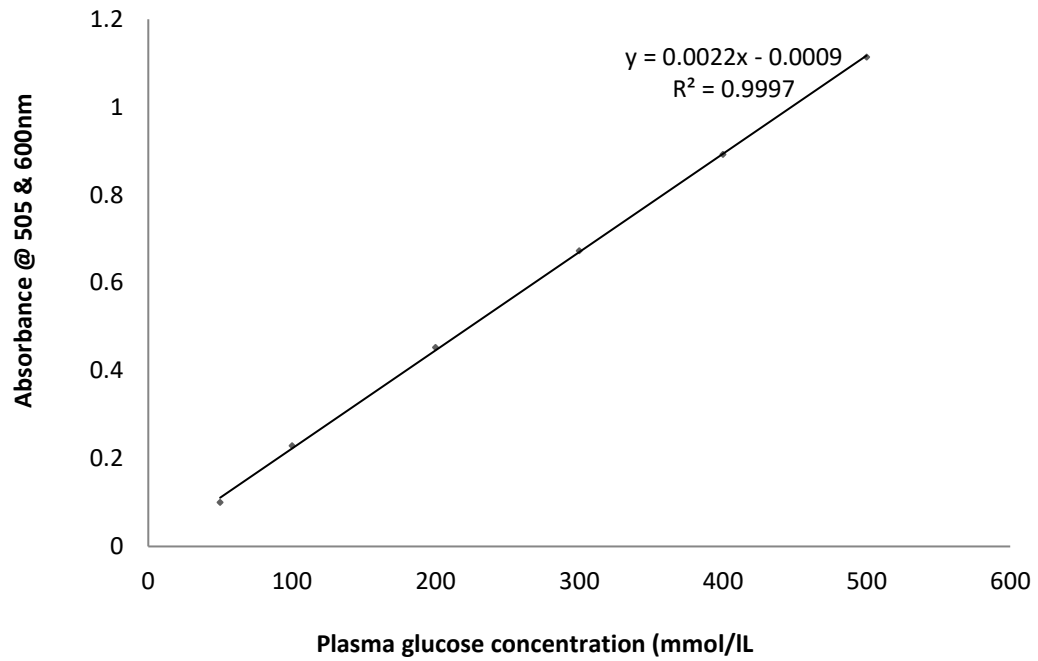
Example calibration curve obtained for plasma insulin concentration



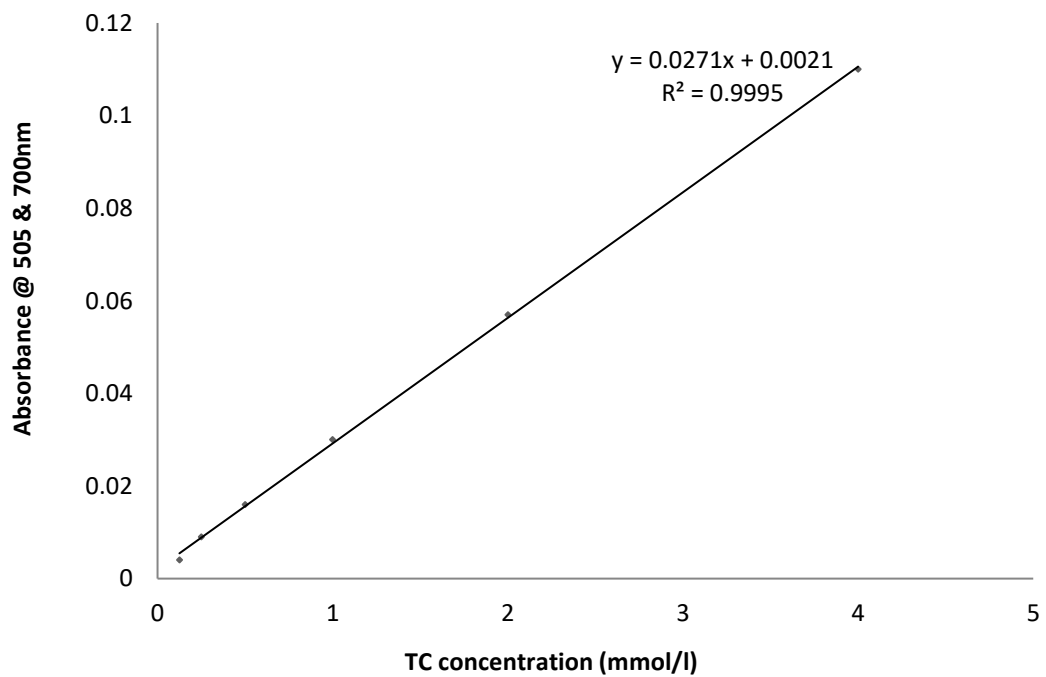
Example calibration curve obtained for plasma hs-CRP concentration



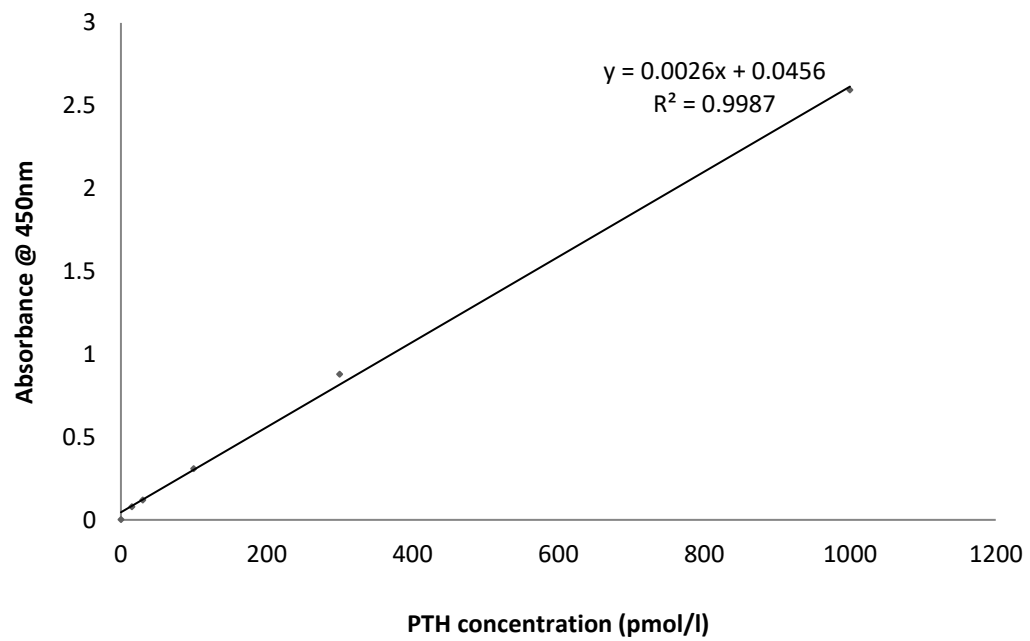
Example calibration curve obtained for plasma HDL-C concentration



Example calibration curve obtained for plasma glucose concentration



Example calibration curve obtained for plasma TC concentration



Example calibration curve obtained for plasma PTH concentration

EFFECT OF VITAMIN D SUPPLEMENTATION ON ENDOTHELIAL AND INFLAMMATORY MARKERS IN ADULTS. A SYSTEMATIC REVIEW OF RANDOMISED CONTROLLED TRIALS. Agbalalah.T., Freeborn.E.J., Hughes. S.F., Mushtaq.S. Department of Clinical Sciences and Nutrition, University of Chester, Chester, UK. CH1 4BJ.

It is evident that vitamin D exerts important pleiotropic effects distinct from its primary role in calcium and phosphate homeostasis, and bone metabolism. Deficient vitamin D status has previously been associated with increased cardiovascular disease risk, therefore the effect of vitamin D supplementation on cardiovascular risk markers, especially endothelial dysfunction and inflammation, are important areas for investigation. In order to investigate the feasibility of vitamin D supplementation as a potential therapy, we systematically reviewed randomised placebo controlled trials (RCTs) investigating the effects of vitamin D (D₂ and D₃) on endothelial function and inflammatory markers in adults. Included studies were identified by conducting a literature search of randomised controlled trial articles published from 2008 to 2014, using Cochrane, Pubmed and Medline electronic databases with the search terms related to vitamin D and endothelial function. Inclusion criteria were RCTs in adult humans with a measure of vitamin D status using serum/plasma 25(OH)D and studies which administered the intervention through the oral route. Among the 1107 studies retrieved, 27 studies met the full inclusion criteria for this systematic review. Overall, eight studies reported significant improvements in the endothelial/inflammatory biomarkers and parameters measured. However, the positive effect disappeared by the second period of measurement in two out of the eight studies. The remaining 19 trial studies did not show significant improvements in the biomarker/parameters of interest measured. Although some studies showed significant improvement in the markers of interest, evidence from most of the studies included in this systematic review did not demonstrate that vitamin D supplementation in adults' results in improvement of circulating inflammatory and endothelial function biomarkers/parameters. RCTs of high methodological quality and a high daily dose (5000IU to 1000IU) of vitamin D are required especially in populations at risk of vitamin D deficiency.